The British Mycological Society

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TRANSACTIONS

PUBLISHED BY
THE SYNDICS OF THE CAMBRIDGE UNIVERSITY PRESS
Bentley House, 200 Euston Road, London, N.W.1
American Branch: 32 East 57th Street, New York 22, N.Y.

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)

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Volume 43

Edited by

S. D. GARRETT and GRACE M. WATERHOUSE

CAMBRIDGE
AT THE UNIVERSITY PRESS
1960

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VOL. 43, PART 1 MARCH 1960

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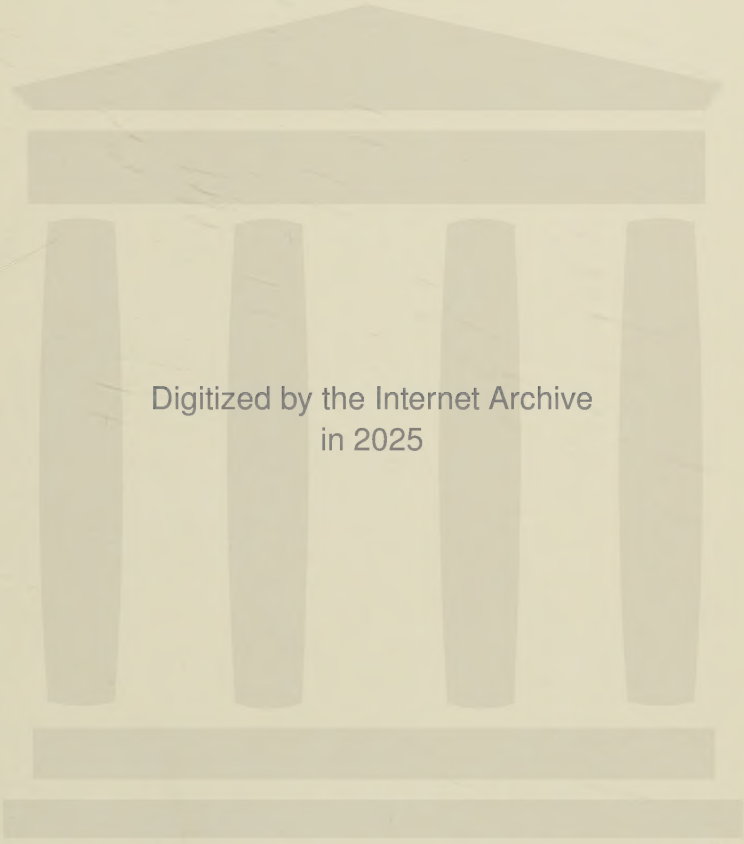
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the germ-tubes. Neither Hemming nor I thought this observation of any great significance and the matter was temporarily forgotten. I suppose our minds were too firmly fixed on the idea that an antifungal substance of interest should prevent spore germination.

Two years later we examined a number of species of *Penicillium* isolated from Wareham Heath soil (Brian, Hemming & McGowan, 1945). Some of these, which we identified as *P. janczewskii* Zal. but which, following the usage of Raper & Thom (1949), I shall refer to below as *P. nigricans* (Bain.) Thom, produced culture filtrates which caused severe stunting and malformation of *Botrytis allii* germ-tubes, and in lower dilutions 'waving' similar to that we had observed in 1942. This made us look back to our earlier results and we found that the original *Penicillium* was also a strain of *P. nigricans*. This time we decided to attempt to isolate this 'curling-factor'. To-day, it seems astonishing that amongst the first fifteen organisms we examined, two produced previously undescribed antibiotics; since then the rate of discovery has declined considerably!

Isolation of the 'curling-factor' was not particularly difficult and a pure active substance was obtained by chloroform extraction of culture filtrates or of mycelium (Brian, Curtis & Hemming, 1946). It was at first thought to contain only carbon, hydrogen and oxygen (McGowan, 1946), but was subsequently shown to contain chlorine as well (Grove & McGowan, 1947) and to be identical with a substance isolated much earlier from the mycelium of *P. griseofulvum* Dierckx by Oxford, Raistrick & Simonart (1939). Thus it was, after all, a known substance, though its effects on growth of fungal hyphae had not previously been recognized. It is now known that several species of *Penicillium* produce griseofulvin. These include *P. urticae* Bain. and the closely related *P. griseofulvum* Dierckx; *P. nigricans* (Bain.) Thom, and probably also a number of very similar species, such as *P. raciborskii* Zal., *P. kapuscinskii* Zal. and some strains of *P. albidum* Sopp., and finally *P. raistrickii* Smith (Brian, Curtis & Hemming, 1955). In our early experiments on surface culture we obtained yields of the order of 20 g. griseofulvin from 50 l. of culture (Brian, Curtis & Hemming, 1949). About 60 % of the griseofulvin was found in the mycelium. More recently, workers in Glaxo Laboratories Ltd. [Brit. Pat. No. 784618] obtained even greater yields of griseofulvin in stirred, aerated cultures. Under such conditions as much as 95 % of the griseofulvin remained in the mycelium unless the fermentation was carried on until autolysis commenced, in which case the griseofulvin was released into the culture medium.

Antifungal activity

We can distinguish under this heading the effect on growth of individual hyphae and the effect on colonial growth. As a convenient example of its effects on hyphal growth we can consider its effect on germ-tubes of *Botrytis allii*. Even in saturated solutions, percentage germination of *B. allii* spores is not reduced. Concentrations of griseofulvin of the order of 5–10 μg./ml. cause severe stunting of germ-tubes, which have a gnarled appearance with swollen or spatulate extremities; after the initial production of this distorted germ-tube, further extension is very slow indeed. In

concentrations of 1 μg./ml. germ-tubes are less severely stunted, branching is unusually abundant, and the hyphae are distorted and lose their usual tendency to grow radially outwards from the spore, frequently turning back on themselves. In still lower concentrations (*c.* 0·1–0·2 μg./ml.) these symptoms are much reduced or absent and a new feature emerges—development of a regular helical curling of the hyphae (Brian, Curtis & Hemming, 1946). Essentially similar effects have been observed in many other fungal species; another feature commonly seen, particularly in *Fusarium* spp., is the production of intercalary, chlamydospore-like swellings

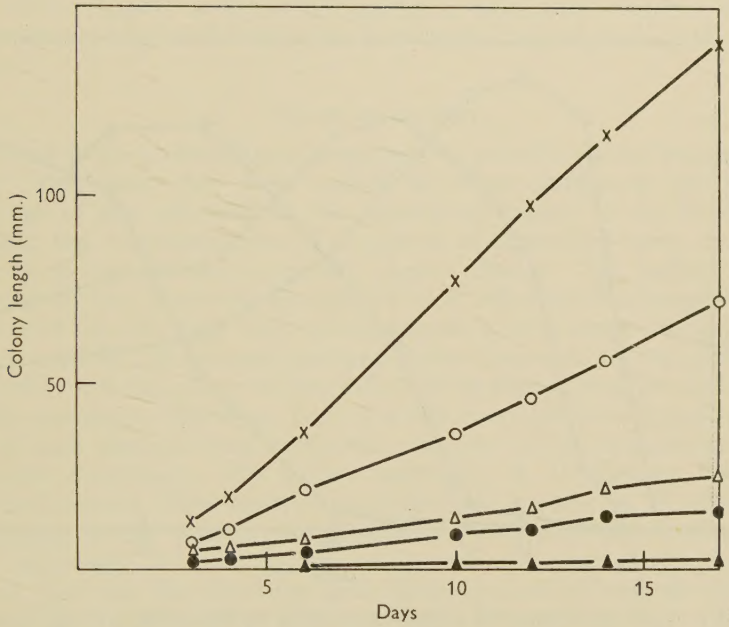


Fig. 1. Length of *Botrytis allii* colonies growing in growth-rate tubes on Czapek agar media (5% glucose) containing 5 μg./ml. (—▲—), 1 μg./ml. (—●—), 0·5 μg./ml. (—△—), 0·2 μg./ml. (—○—) and no (—×—) griseofulvin.

(Brian, 1949; Napier, Turner & Rhodes, 1956; Aytoun, 1956). *Botrytis allii* is among the more susceptible fungi to griseofulvin; others may show only slight morphological anomalies even in saturated solutions and others appear to be completely resistant. This variation in susceptibility is discussed further in a later paragraph. Though other substances are known which will cause hyphal distortion, generally speaking none are so active as griseofulvin in low dilutions. Moreover, the effects are never precisely similar and I know of no substance other than griseofulvin and a few related compounds which produce helical curling of fungal hyphae, perhaps the most characteristic response to griseofulvin. The unique character of this response has made it possible to develop a simple but effective bioassay for griseofulvin, using *Botrytis allii* as the test organism.

As a result of these abnormalities of hyphal growth, the radial growth of

fungal colonies on agar media containing griseofulvin may be severely restricted. Radial growth of *B. allii* colonies is reduced to a negligible level by 5 μg./ml. griseofulvin; this is illustrated in Fig. 1, which shows the rate of spread of *B. allii* colonies on agar media in growth-rate tubes (Ryan, Beadle & Tatum, 1943). Other fungi require a larger dose of griseofulvin to produce the same effect; a few, as I shall mention later, are even more sensitive than *B. allii*. Colonies of *B. allii* on griseofulvin agar have been kept under observation for several weeks, but no tendency for colonies or sectors of colonies to overcome the inhibition has ever been noticed and the growth rate remains fairly constant.

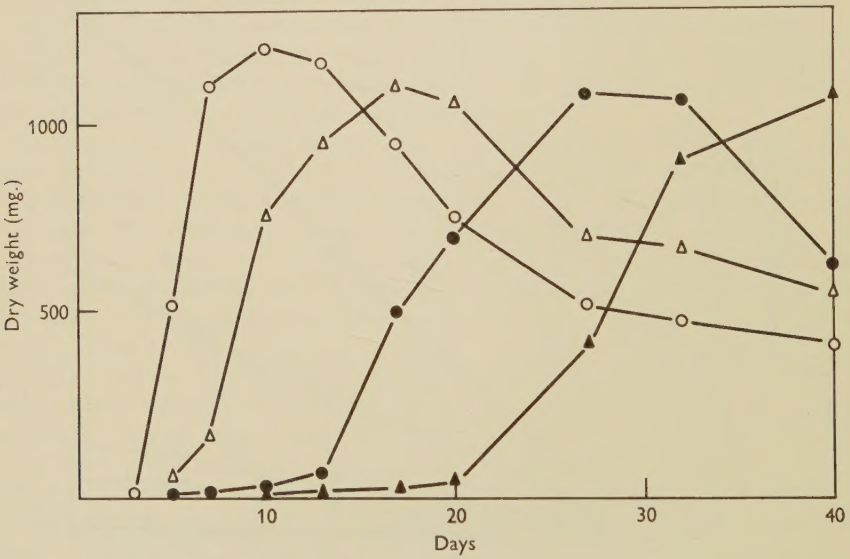


Fig. 2. Dry weight of *Botrytis allii* cultures growing as surface cultures at 25° C. on liquid Czapek media (5% glucose) containing 10 μg./ml. (—▲—), 5 μg./ml. (—●—), 1 μg./ml. (—△—) and no (—○—) griseofulvin.

Radial growth of colonies is only one of several possible measures of fungal growth; another is dry-weight accumulation. We have measured the effect of griseofulvin on dry weight of surface colonies of *B. allii* on liquid media (Text-fig. 2). Initially, 5 μg./ml. griseofulvin is as inhibitory as in agar media, but after about 3 weeks the growth rate increases considerably. If mycelium from such cultures is then used to inoculate fresh griseofulvin-containing media, the initial lag period is much reduced and after several such transfers quite rapid growth can be obtained on media containing 20 μg./ml. griseofulvin. However, the growth rate never reaches that of cultures on griseofulvin-free media, and, in detail, hyphal distortions are still obvious. Nevertheless, it is clear that appreciable adaptation takes place. Mycelium from such adapted colonies will grow freely on agar media containing 20 μg./ml. griseofulvin; inocula from unadapted colonies do not grow at all. The adaptation is rapidly lost if

mycelia are transferred back to griseofulvin-free media for a short time (Brian, Elson & Hemming, unpublished results). Such adapted mycelia produce few viable spores, so we have not been able to determine whether the adaptation is transmitted through the spore. The rapid loss of adaptation on removal of the inhibitor is reminiscent of the results with other inhibitors recently reported by Bartlett (1959), and in this case, as in his experiments, it seems unlikely that the adaptation is based on mutation.

I should add that we have found that when growth of mycelia does commence on griseofulvin media there is a progressive loss of griseofulvin from the medium; this loss is a consequence of fungal growth, not the cause of it. Abbott & Grove (1959) have presented evidence that the disappearance of griseofulvin is due to enzymic degradation on the hyphal surface.

Mode of action

The nature of the morphological responses to griseofulvin led me to suggest (Brian, 1949) that they were caused by some change in the physical properties of the cell-wall in the extending region of the hyphal tip, involving the transformation of a system of organized local change in plasticity to one of more general plastic change. The helical waving produced by low doses was thought to be a reflexion of a tangential fine structure of the cell-wall, such as is known to exist in some fungal hyphae. Hyphal walls laid down and matured before exposure to griseofulvin are unaffected; it is the zones of new cell-wall formation that are influenced. We may envisage, therefore, that it is the synthesis and organization of new cell-wall material that is affected. Aytoun (1956) has interpreted his observations similarly. My former colleague, K. D. Cooper, has made some preliminary time-lapse cinematographic studies of *B. allii* spores germinating in the presence of griseofulvin, and these give a very strong visual impression that the rigidity of the cell-wall near the hyphal tip is reduced and that the swellings and distortions arise from the failure of these weaker membranes to withstand internal pressures.

Indirect support for this interpretation of the response to griseofulvin as a 'cell-wall effect' is afforded by the association of response to griseofulvin with cell-walls of a specific chemical nature. None of those fungi with cell-walls based on cellulose, e.g. the Oomycetes, are distorted by griseofulvin. All those fungi which are distorted by griseofulvin are ones in which chitin is, or in all probability is, a major structural constituent. Though it is true that a few such species, of the many tested, show no response to griseofulvin (Napier *et al.* 1956), there is a strong general correlation between response and the presence of chitin in the cell-wall. This tempts one to suggest that griseofulvin may produce its effects by somehow interfering with biosynthesis of the chitinous elements of the cell-wall.

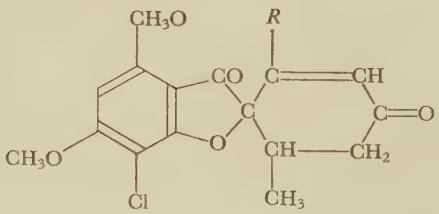
Also of interest in this connexion are certain observations made by Banbury (1952) in the course of his studies of phototropism in the *Phycomyces* sporangiophore. He was interested in the possibility that an explanation of the tropic curvatures of this organ could be found in terms of an altered distribution of a growth-regulating hormone, by analogy with the Cholodny-Went theory of tropism in higher plants. Unilateral applica-

tions of auxins, such as indolylacetic acid, to the sporangiophore had no effect, though, as you well know, bending of coleoptiles can be induced in that way. However, Banbury did find that griseofulvin would induce curvatures. Now it would be extravagant to press the suggestion that griseofulvin is a natural fungal hormone, but taken together with the other evidence that I have already mentioned, the observation does support the view that griseofulvin somehow intervenes in the mechanism regulating cell-wall growth.

A further characteristic of griseofulvin action can be conveniently mentioned at this point. It only affects hyphae and hyphal regions with which it is in immediate physical contact; there appears to be no translocation within the hypha. If *Syncephalastrum racemosum* is growing on agar containing griseofulvin the hyphae growing in the medium are profoundly distorted, whereas the aerial stolons, characteristic of the species, grow quite normally (Brian, 1949), and aerial hyphae of *Botrytis* spp. in similar conditions are also unaffected (Aytoun, 1956). Banbury (1952) found that griseofulvin would only cause bending of *Phycomyces* sporangiophores if applied directly to the apical growing zone, and was without effect if applied a few millimetres below. Abbott & Grove (1959), using a spectrophotometric technique, have been unable to demonstrate any uptake or translocation of griseofulvin by *Phycomyces*; indeed they were unable to detect any accumulation of griseofulvin or metabolites of griseofulvin in hyphae of *Phycomyces blakesleeanus*, *Botrytis allii* or *Mucor ramannianus*. It therefore seems possible that the morphogenetic responses to griseofulvin are the result of an effect on processes taking place in the cell-wall itself and that the griseofulvin is acting 'from the outside' of the cell.

Biological activity and molecular structure

Grove, MacMillan, Mulholland & Rogers (1952) have shown griseofulvin to have the structure I ($R = OCH_3$).



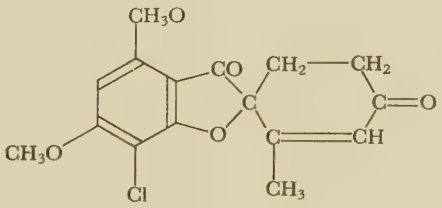
I

As I mentioned earlier, the griseofulvin molecule contains a chlorine atom. We therefore studied the effect of using chlorine-free media on the development of 'curling-activity' in culture filtrates. To our surprise we found that, despite most rigorous purification of medium constituents, 'curling-activity', though reduced, was never by any means eliminated. At first we suspected that our methods of purification were at fault, but the true explanation was found later. Particularly in chlorine-deficient media, but also to some extent in chlorine-containing media, *Penicillium griseofulvum*

and *P. nigricans* produce a compound closely related to griseofulvin—dechlorogriseofulvin—in which the chlorine atom is replaced by a hydrogen atom. This has typical griseofulvin-like activity (MacMillan, 1953). If, instead of adding potassium chloride to media, potassium bromide is added, a bromo-analogue of griseofulvin is formed (MacMillan, 1954). This was the first bromine-containing fungal or bacterial metabolite to be characterized. The least concentrations (μg./ml.) of these compounds causing helical waving of hyphae of *Botrytis allii* are as follows: griseofulvin, 0·1; bromo-analogue of griseofulvin, 0·75; dechlorogriseofulvin, 6·25.

By modification of the griseofulvin molecule more active compounds can be obtained. The *n*-propyl and *n*-butyl homologues (*I*, R = OPr^n and OBu^n respectively) cause helical waving at concentrations less than one-twentieth of the minimal effective concentration of griseofulvin (Crowdy, Grove & McCloskey, 1959).

Finally, some griseofulvin relatives with typical 'curling-activity' have now been synthesized. I will mention only one. The compound with structure II (Dawkins & Mulholland, 1959) causes helical waving of *B. allii* hyphae at a concentration of 3·1 μg./ml.; thus



II

though less active than griseofulvin, it is not a lot less active.

Systemic distribution in plants

The soil microflora can affect the growth of plants in various ways. Root disease, mycorrhizal associations, and release of nutrients from sources unavailable to higher plants, are among the topics that come to mind in this connexion. But it has for long seemed to me an intriguing possibility that organic molecules synthesized by the soil microflora might pass directly into plant roots and thence into the aerial organs, possibly with important consequences. Until recently little has been known of the uptake of such molecules by roots. Because of its relatively low toxicity to plants (Brian, 1949; Wright, 1951) and the possibility of detecting it reasonably specifically in low concentrations, griseofulvin seemed a useful substance with which to do a little preliminary experimental work. Oat seedlings were grown in water culture and griseofulvin was added to the culture solution. At various intervals after the beginning of the experiment the plants were placed in a humid atmosphere to encourage guttation, and samples of the guttation fluid were taken and assayed for griseofulvin. This simple and convincing technique for the detection of uptake and translocation had been developed by Frohberger (1948) in work on systemic insecticides. 'Curling-activity' could be detected in the guttation drops

a few days after application to the roots. Similar results were obtained with lettuce and tomato seedlings; with these plants the guttation technique could not conveniently be used, but 'curling-activity' was detected in aqueous leaf extracts (Brian, Wright, Stubbs & Way, 1951). Though relatively non-phytotoxic, griseofulvin did cause some damage to plants. Of those with which we worked the plants most susceptible to damage were the cereals. Tip-burn of leaves was common, almost certainly a consequence of accumulation of griseofulvin in that region. A more interesting effect was a swelling of the roots (Stokes, 1954). This effect is probably associated with the interference with mitosis in root apices by high concentrations of griseofulvin subsequently demonstrated by Paget & Walpole (1958). On the other hand, Rhodes (1955) has reported increased vigour of carnation plants treated with this antibiotic.

Having found that griseofulvin could safely be introduced into the tissues of plants, we were inevitably led to consider whether such a systemic treatment would confer resistance to fungal diseases. In a few simple experiments we found an appreciable degree of control of cereal mildews, grey-mould (*Botrytis*) on lettuce, and of *Alternaria solani* on tomato. These encouraging preliminary results have been followed by a series of detailed studies of the uptake of griseofulvin and its fate in plant tissues, and of the value of griseofulvin as a 'fungicide' in practice.

I shall first deal with the translocation and fate of griseofulvin in the plant, basing my remarks mainly on the work of Stokes (1954), Crowdy, Gardner, Grove & Pramer (1955) and Crowdy, Grove, Hemming & Robinson (1956). This work has been concerned solely with uptake through the roots. Under such conditions uptake and translocation of griseofulvin involves at least two processes. The passage of griseofulvin from the root surface to the vascular system of the root is a complex process; it is rapid, independent of transpiration and probably linked with respiration. Translocation from the root vascular system to the shoot involves a passive movement of the compound with the transpiration stream. The amount of griseofulvin taken up by a plant is linearly related to the amount of water transpired; in other words, if you are working in a naturally variable environment, the dose you actually give to the plants by root application is determined by those environmental factors which govern the transpiration rate, viz. leaf-temperature, stomatal aperture, atmospheric humidity, air movement and availability of water to the root.

A question that will always be of importance in systemic treatment is—Is the substance chemically modified in the plant tissue? Griseofulvin does appear to be absorbed and translocated substantially unchanged in short-term experiments, and to prevent development of fungal pathogens by its characteristic effect on hyphal extension. This statement is based on three kinds of evidence: (*a*) the possibility of extracting material with 'curling-activity' from leaves; (*b*) the observation of Aytoun (1956) that *in vivo* inhibition of *Botrytis tulipae* in tulip stem tissue is associated with typical morphological disturbances in the fungal hyphae; (*c*) direct chemical evidence, involving recovery and identification of griseofulvin from the leaves of plants growing in a culture solution containing the antibiotic. However, some chemical modification of griseofulvin does also take place.

For example, in an experiment where bean seedlings were grown for 3 days in a solution containing 123 mg. griseofulvin, 82 mg. remained in the solution, 20 mg. were found in the roots and 11 mg. in the shoots. This left 10 mg. unaccounted for. Subsequent work showed that this was due to a breakdown of griseofulvin in the plant tissues, the 'half-life' of a quantity of griseofulvin in bean tissue, in these experimental conditions, being about 4 days. Experience has shown that a single dose of griseofulvin gives a protective effect for only a limited time; this is most probably due to the sort of breakdown I have described. I should add that there is every indication that the half-life varied from one plant species to another.

Very little work has been done on translocation of griseofulvin applied to the foliage of plants, though this would be the preferred method of application. Because of the great importance of the link between transpiration and the movement of griseofulvin, one would expect less mobility when applied to the leaves. However, local translocation within leaf-tissues does take place (Davis & Rothrock, 1956).

A very interesting study of the movement of a series of griseofulvin homologues and relatives, and of their efficiency as systemic protectants against fungal attack, has been made by Crowdy, Grove & McCloskey (1959). I should like to extract one observation from their work. The *n*-butyl and *n*-propyl homologues of griseofulvin are, as I mentioned earlier, far more active than griseofulvin itself *in vitro*, but were found to be completely ineffective as systemic 'fungicides'. This was shown to be due to accumulation in the root, scarcely any being passed on through the transpiration stream to the shoot. The failure to move from the root was attributed to the low water-solubility of these compounds, coupled with a high oil:water partition coefficient, leading to binding in a lipoid phase in the root tissues. This emphasizes the important point that desirable properties of a systemic fungicide not only include activity *in vitro*, stability and lack of phytotoxicity, but also the correct water-solubility and oil:water partition properties to ensure translocation.

Production of griseofulvin in soil

Of the fungi which produce griseofulvin, one, *Penicillium nigricans*, is a common soil-saprophyte. Would it be possible to induce this to produce griseofulvin biologically near the root surface of plants, and so protect both roots and shoots against fungal attack? This does not seem to be feasible. Not only is griseofulvin quite rapidly biologically degraded in soil (Jefferys, 1952; Wright & Grove, 1957), but *P. nigricans* appears to be very sensitive to the presence of other organisms and griseofulvin synthesis other than in pure culture is negligible (Wright, 1955).

Griseofulvin as an agricultural 'fungicide'

Despite the encouraging early results, griseofulvin does not, on the whole, show great promise in practical control of plant disease. It will undoubtedly give a degree of control of quite a number of plant diseases. It is quite effective against a number of mildews including the powdery mildew of

roses caused by *Sphaerotheca pannosa* (Kirby, 1957), *Pseudoperonospora cubensis* on cucumber (Ark & Thompson, 1957), and *Oidium chrysanthemi* on chrysanthemum (Rhodes, Crosse, McWilliam, Tootill & Dunn, 1957), but is quite ineffective against *Sphaerotheca humuli* on strawberries (Ingram, Schofield & Taylor, 1957). It has controlled some *Botrytis* diseases, including grey mould of lettuce and tulip fire (Rhodes *et al.* 1957) and turf diseases caused by *Fusarium nivale* and *Sclerotinia homoeocarpa* (Drew-Smith, 1957). A surprising result, because griseofulvin has never been shown to possess antibacterial activity *in vitro*, is a considerable degree of control of artificially induced blossom blight (*Erwinia amylovora*) of pears (Hamilton & Szkolnik, 1957). It gives some control of club-root of *Brassicae* (Campbell, 1956; Potter, 1956; Rich, 1957; Rosser, 1957), but an early observation suggesting that it would control club-root systemically if applied to the foliage has been shown to be mistaken (Potter, 1957; Rich, 1957). However, though griseofulvin affords some control of a number of plant diseases, it has usually been found to be no better than, or inferior to, other fungicides already in general use. One of the few cases where griseofulvin is apparently more effective than other fungicides is in control of the brown rot of apples caused by *Sclerotinia fructigena*. Byrde (1959) has shown a significant reduction of infection in three successive seasons after application of griseofulvin (0·05–0·1 %) as an aqueous spray.

Control of animal mycoses

Griseofulvin is very effective *in vitro* against several dermatophytic fungi. In my experience colonial growth of *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum canis* is almost completely inhibited by 1 μg./ml. griseofulvin in agar media; these fungi are even more sensitive to the antibiotic than is *Botrytis allii*. It is particularly interesting, therefore, that griseofulvin possesses systemic activity against ringworm. Oral treatment has been shown to cure experimental infections of guinea-pigs with *Microsporum canis* and *Trichophyton mentagrophytes* (Gentles, 1958; Martin, 1959), and experimental infection of cattle with *T. verrucosum* (Lauder & O'Sullivan, 1958). It has been used successfully clinically on man, by mouth, in cases of infection of finger-nails or skin with *Trichophyton* spp. and of scalp infection with *M. canis* and *M. audouinii* (Williams, Marten & Sarkany, 1958; Blank & Roth, 1959; Cochrane & Tullett, 1959; Beare & Mackenzie, 1959; Barlow, Chattaway, Hargreaves & La Touche, 1959). No evidence of toxicity was observed in these experiments. The toxicity to mammals is very low, when taken by mouth, and the only report of any untoward effect is that large doses given intraperitoneally or intravenously to rats have caused some nuclear disturbance in bone-marrow and seminal epithelium (Paget & Walpole, 1958). In the animal, as in the plant, it appears that griseofulvin produces its antifungal effect at the site of action by the same mechanism as *in vitro*. Fungi attempting to invade hairs after oral treatment with griseofulvin show characteristic hyphal malformations, and material with griseofulvin-like activity, as determined by the *B. allii* bioassay, can be extracted from the hair of orally-treated animals (Gentles, Barnes & Fantes, 1959).

Topical treatment of skin and nail infections with fungicides has never given completely satisfactory results. With the exception of a single report of successful oral treatment of trichophytiasis with salicylhydroxamic acid (Alkiewicz, Eckstein, Halweg, Krakowka & Urbanski, 1957), this work with griseofulvin is the first indication that systemic treatment of superficial mycoses is possible. Thus griseofulvin may find a useful application in medical and veterinary practice.

Conclusion

No research is completely valueless. Even though griseofulvin may probably never be used to any extent as an agricultural fungicide, and though its future in control of animal mycoses is still undecided, though decidedly promising, I think it can be claimed that the investigations I have mentioned here have some lasting scientific value. In the first place, I think they have proved the practical possibility of systemic control of fungal diseases of plants and animals; we now know that substances can be found with the necessary specificity of toxic action. Secondly, I think griseofulvin will prove to be a useful tool in the study of the metabolism of the hyphal cell-wall. Hitherto this field of research has been much neglected, yet knowledge of the process is essential if we are to understand growth or regulation of growth of either simple or complex fungal organs.

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STUDIES ON BRITISH CHYTRIDS. XV. *CHYTRIDIUM COLEOCHAETES* NOWAKOWSKI

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(With 3 Text-figures)

Rhizophidium coleochaetes (Nowak.) Fischer has been rediscovered and the presence of a tubular rhizoid and an operculum demonstrated. The fungus thus appears closer to *Chytridium* than to other genera in which it was subsequently placed.

Introduction

Nowakowski (1876) described *Chytridium coleochaetes* parasitizing the oogonia of *Coleochaete pulvinata* A.Br. from Germany. No further record of this fungus has been discovered in the literature and, owing to the lack of certain details in the original description, its generic position has remained in doubt. Indeed it was placed in *Olpidium* by Schroeter (1885) and in *Rhizophidium* by Fischer (1892), but from the account presented here it is clear that Nowakowski's name must stand.

In August 1958 the chytrid was rediscovered by Dr B. Plunkett in oogonia of *Coleochaete pulvinata*, growing on *Equisetum* stems in Moss Eccles Tarn, Claife Heights, Lancs. He kindly gave me the material and further collections were made until the *Coleochaete* disappeared at the end of November. Unfortunately specimens were never common and its life history still remains incomplete. However, since a locality for it has now been found future collections and studies can be made.

Life history

The zoospore passes down the open trichogyne (Fig. 2*a*) and comes to rest on the walled oosphere. Early stages in development, especially of the endobiotic system, are most difficult to see. This is due to the dense green content of the oosphere and to the mucilage surrounding the alga, which hampers decolorization. The earliest stage in thallus development is shown in Fig. 2*b* and closely resembles that pictured by Nowakowski (Fig. 1*a*). The delicate-walled tube-like sporangium, containing minute non-refractive granules, is growing up the trichogyne, and inside the oosphere is a large gleaming globule (*x*, Fig. 2*b*), which no doubt indicates the position of the rhizoid. The latter varies in shape from tubular, lunate or irregular, 13–17 $\mu \times 5$–$5{\cdot}5$ μ, to almost spherical, 14 μ diameter. No branched specimens have been found nor the presence of any other rhizoids. In the natural state the rhizoid is never clearly visible until the sporangium is well developed and the host content has shrunk (Fig. 2*g*, *h*, *j*–*l*). By the

time the sporangium is empty the rhizoid (possibly because of its thin wall) is once again invisible so that it is not surprising that Nowakowski failed to find it. It is sometimes possible to demonstrate the rhizoid in young stages after decolorization of the alga in hot glacial acetic acid (Fig. 2*c*, *f*). The rhizoid is connected to the base of the sporangium by a narrower portion (Figs. 2*f*, *i* and 3*f*). The sporangium (Fig. 2*h–l*) is, as described by Nowakowski, unequally spindle shaped, 47–106 μ high and 11 μ at its greatest diameter which is the portion just beyond the trichogyne. It narrows at the apex where an operculum develops, and basally there is a foot-like portion of very varied extent. Sometimes this latter part is almost non-existent (Fig. 2*f*), at other times it is elongate, broad (Fig. 2*h*, *k*).

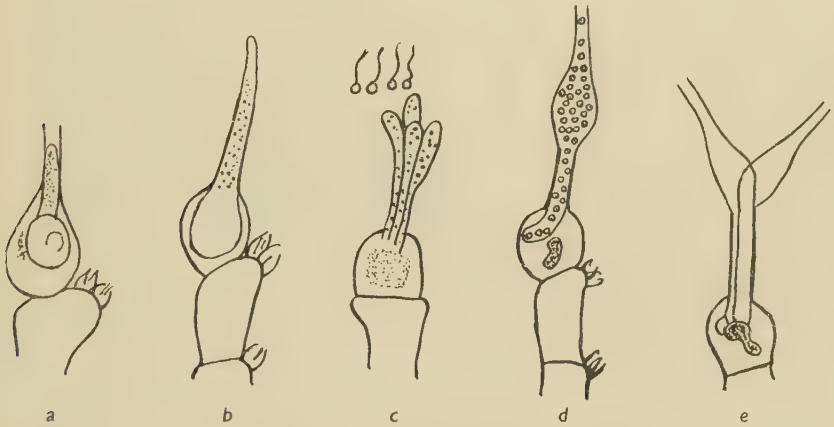

Fig. 1. *Chytridium coleochaetes* after Nowakowski (1876), ×400.

or narrow (Fig. 3*c*). It can also be recognized in Nowakowski's figure (Fig. 1*d*). I believe this portion is situated between the walls of the oogonium and oosphere, but how it develops is unknown. It could be that in specimens with a large 'foot' the zoospore has bored deeply between the two walls before coming to rest and germinating; or that a zoospore settles at the base of the trichogyne and then produces a germ thread of some length before penetrating the oosphere. The development of such a sporangium would represent both the expansion of the germ thread and the zoospore cyst. This might happen if an oogonium were infected immediately after fertilization and when its neck had become cut off by a septum. No signs of cortification have ever been observed. Again, since a sac-like endobiotic rhizoid is formed there remains the possibility of endoexogenous development, but I think this is most unlikely. Occasionally, abnormally shaped sporangia were found (Fig. 3*h*, *i*). Changes in the protoplasm leading to the formation of zoospores are similar to those in most chytrids except that a vacuolate stage has been noted (Fig. 2*g*). The convex operculum is distinguishable while the sporangium is still immature (Fig. 2*h*, *i*); it becomes more refractive as maturity approaches. On dehiscence the lid is cast aside and a few zoospores, 2·5 μ diam., and containing a bright globule, emerge fully formed in a single file through

Fig. 2. *Chytridium coleochaetes* Nowak. *a*, Zoospore in trichogyne; *b*, young thallus with endobiotic refractive globule (*x*); *c–i*, stages in growth and maturation of sporangia; *g*, protoplasm in vacuolate stage; *j–l*, mature sporangia; *m*, apical part of a sporangium about to dehisce; *n*, *p*, dehisced sporangia containing zoospores apparently with no flagellum; *o*, opercula. In *c*, *f–h*, *j–l*, the rhizoidal sac is visible. Figs. *c* and *f* mounted in glacial acetic acid. All × 650.

the narrow apex. A flagellum, 15–25 μ long, is present but the zoospores are usually rather sluggish at this stage; active free-swimming zoospores have, however, been observed.

Fig. 3. *Chytridium coleochaetes* Nowak. *a–e*, Empty sporangia; *f*, *g*, sac-like rhizoid; *h*, *i*, abnormal sporangia; *j*, two sporangia with rhizoids, mounted in glacial acetic acid; *k–n*, unidentified hyperparasite; *k*, *l*, immature, and *m*, *n* empty sporangia. All ×650.

The emptying of a large sporangium which contains well over 100 zoospores may take a long time and it is not uncommon to find incompletely dehisced specimens. Often they contain quiescent zoospores apparently with no flagellum (Figs. 1*d*, 2*p*): whether these zoospores do eventually emerge or die is unknown. As shown by Nowakowski (Fig. 1*c*), up to four sporangia may occur in a single oogonium and the content of the latter is reduced to a characteristic reddish brown residue.

Sporangia of *Chytridium coleochaetes* may be infected by an unidentified hyperparasite. The sporangium is top-shaped, 9–18 μ high × 5–12 μ wide, with a short stalk and a richly branched endobiotic rhizoidal system (Fig. 3*k–n*). Empty sporangia are vase-shaped with a wide apical opening (Fig. 3*m*, *n*), suggesting the presence of an operculum. Of known hyperparasites, it most nearly resembles *C. parasiticum* Willoughby (1956), and in its vase-shaped empty sporangium it greatly simulates *C. lecythii* (Ingold) Goldie-Smith (1946).

The resting spore of *C. coleochaetes* remains unknown. It was not seen by Nowakowski and none was identified with certainty from the Moss Eccles material. The only possible specimen was seen while photographing the fungus and no drawing of it was made. It was a thick-walled spherical body containing a single large refractive globule and situated within the oosphere.

Now a locality for this fungus is known future collections can be made to elucidate the many puzzling aspects of its life history and to observe the resting spore. However, from this investigation it seems that Nowakowski's original binomial, *Chytridium coleochaetes*, is at present the most appropriate.

My thanks are due to the members of the Laboratory Staff who collected samples and to Dr J. W. G. Lund for reading the manuscipt. This investigation was carried out with apparatus purchased from a grant made to me by the Central Research Fund of the University of London.

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(*Accepted for publication* 2 *March* 1959)

Trans. Brit. mycol. Soc. **43** (1), 19–30 (1960).

THE ECOLOGY OF SOME AQUATIC PHYCOMYCETES

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(With Plate 1 and 4 Text-figures)

The data presented here suggest that the 'rarity' of less commonly recorded species of aquatic phycomycetes often appears to be due, not simply to specificity of substratum and toleration of only a limited range of hydrogen-ion concentration, but also to temperature requirements and the time needed for maturation of the resting spores. As a result of these last two factors there is a distinct seasonal periodicity of growth, mainly restricted to spring and autumn. These observations are based on the results of an investigation of the phycomycete flora of four groups of collecting stations, selected from a variety of freshwater habitats in North Staffordshire, Cheshire and South Wales.

The development of suitable methods of collection and subsequent treatment of material (maintenance at 3° C.) made it possible to obtain plentiful growths on natural substrata of many rarely collected phycomycetes. It was shown by experiment that at temperatures above 3° C. many of these delicate and often slow-growing fungi are unable to compete with other organisms present in untreated pond-water.

The forty-four species recorded belong to the Blastocladiales, Monoblepharidales, Plasmodiophorales, Saprolegniales, Leptomitales, Lagenidiales, and Peronosporales. The Chytridiales are excluded, and particular reference is made to members of the Monoblepharidales.

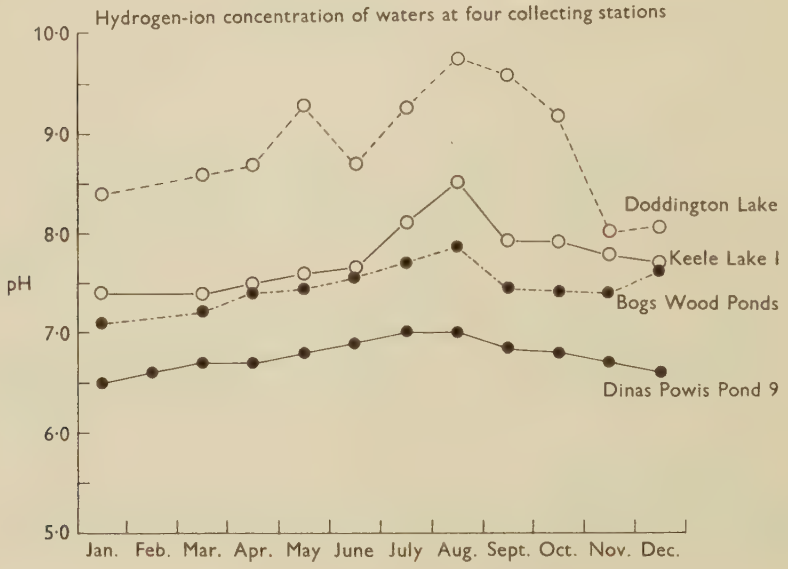
Introduction

As a result of a preliminary survey of the phycomycete flora of a large number of freshwater habitats in N. Staffs. and Cheshire from October 1953 to September 1954, three groups of collecting stations, representing a variety of habitats, were selected for further investigation during the next 3 years. The waters of these collecting stations proved to be either constantly alkaline or neutrally alkaline, according to Lund's (1934) classification of aquatic habitats of phycomycetous fungi, which is used throughout this investigation; therefore a fourth situated in South Wales and having neutrally acid waters was included.

From October 1954 to December 1955 regular monthly collections of a variety of substrata were made from each station, water temperatures were recorded daily, and the pH of the water tested monthly (Text-fig. 1). Supplementary collections which confirm the original data were made during 1956 and 1957.

The purpose of this investigation was in the first instance to determine the composition of the phycomycete flora in a variety of freshwater habitats, with a consideration of some of the factors affecting the growth of these fungi in their natural environment, and secondly to obtain material of species of *Monoblepharis*, on which systematic and morphological studies had previously been made (Perrott, 1955), with the object of explaining

their infrequent collection, and possibly providing useful information for pure culture experiments. For despite repeated attempts *Monoblepharis*, when isolated in pure culture on an artificial medium, had produced only a meagre growth of vegetative mycelium (Perrott 1955).



Text-fig. 1. Hydrogen-ion concentration of waters at four collecting stations. O—O, O- - -O, Constantly alkaline waters (Lund); ●·—·—●, neutrally alkaline (Lund); ●—●, neutrally acid (Lund).

The habitats studied

(1) Two lakes at Keele, N. Staffs. (Nat. Grid Ref. 33/822446). Each lake is approximately two acres in extent, is situated in parkland on Carboniferous sandstone, and is overhung by a variety of trees. The waters of these lakes have a pH range of 7·4–8·5. This puts them in Lund's constantly alkaline group (Text-fig. 1).

(2) Doddington Mere, a lake of 10 acres at Doddington, Cheshire (Nat. Grid. Ref. 33/714465). This is situated in parkland on a glacial drift of Boulder Clay with overlying sands and gravel, and is overhung by trees, chiefly oak and elm. The waters are constantly alkaline with a pH range of 8–9·7 (Text-fig. 1). There is appreciable wave action on its shores.

(3) A series of small stagnant ponds at Bogs Wood, Keele, N. Staffs (Nat. Grid Ref. 33/818457). These are situated in a small wood on Carboniferous sandstones and do not normally dry up in summer. Their waters are neutrally alkaline with a pH range 7–7·7 (Text-fig. 1).

(4) Three ponds (nos. 5, 9, 15) situated in low-lying meadows on Triassic marls at Dinas Powis, 5 miles west of Cardiff, S. Wales (Nat. Grid Ref. 31/147702). The waters of these ponds fall into Lund's neutrally acid group and have a pH range of 6·4–7. Ponds 5 and 15 occasionally dry up

during the summer months, and pond 15 is often trampled by cattle. All are overhung by trees (Text-fig. 1).

In addition to these stations where regular monthly collections were made, sporadic collections were also made from ponds at Styal, Chesh. (Nat. Grid Ref. 33/835842), Ullswater and Blelham Tarn in Westmorland, Rudyard Lake in N. Staffs. (Nat. Grid Ref. 33/948600), pools in the vicinity of Bristol (Nat. Grid Ref. 33/835842) and Lydney, Gloucestershire (Nat. Grid Ref. 31/620035), and Loch Lomond in Scotland.

Methods of collection and treatment of material

The great majority of aquatic phycomycetes rarely appear in sufficient quantity in nature to be collected as one might obtain algae from a 'bloom'. Being either parasites or saprophytes, often with relatively specialized nutritional requirements, these fungi must be sought on particular hosts or substrata. Therefore a wide variety of baits was used, such as grapes, tomatoes, plums, apples, rose hips and hawthorn berries, twigs of oak, elm, ash, birch, sycamore, alder and larch, and fish, meat and hair, the same range being used each month and at each station. Hemp seeds, which are excellent baits for members of the Saprolegniales, were not used. It was decided that the identification of large numbers of species of *Saprolegnia* and *Achlya* would make the task unmanageable, especially as it was hoped to devote particular attention to less commonly recorded species. Baits were placed in perforated galvanized metal containers with weights attached and immersed in water not more than 1 ft. deep.

After 4 weeks immersion the baits were placed in polythene bags or jars and taken to the laboratory as soon as possible after collection. There they were placed in sterile glass dishes with loosely fitting glass covers. The cover was supported on the rim of the dish by pieces of rubber tubing to allow air to enter. Sufficient distilled water to cover the baits was added and the dishes placed in a refrigerator at 3° C., being brought into the laboratory only for examination.

After an interval mycelium could be seen developing on the baits. By repeated washings with sterile distilled water and the selection of isolated growths, it was often possible to obtain many rarely collected phycomycetes on natural substrata. The constant low temperature appears to reduce the growth of many of the larger, less delicate phycomycetous fungi, e.g. members of the Saprolegniaceae, which tend, at laboratory temperatures (approximately 20° C.) to crowd out the slow-growing and more delicate species, e.g. of *Monoblepharis*. It also seems likely that at temperatures above 3° C. many of these delicate and often slow-growing phycomycetes are unable to compete with bacterial contaminants. In this connexion it is interesting to note that in experiments with pure cultures of *M. sphaerica* Cornu it was subsequently shown that the optimum temperature for hyphal growth is 20° C. (Perrott, 1958), and that vegetative hyphae grow from agar disks containing hyphal tips equally well in autoclaved pond water, Seitz-filtered pond water and sterile distilled water. On the other hand, in untreated pond water hyphal growth compares favourably with growth in the other types of water only at 3° C. At

temperatures above 3° C. growth in untreated pond water is considerably less than growth in sterile water and soon ceases.

Fish and meat proved unsuccessful baits because they soon became heavily colonized by bacteria. Under such conditions the growth of even the stronger growing fungi was checked and sexual reproductive organs were rarely formed.

Mycelial growths were rarely found on adult human hair, and were always vegetative. It appears, however, from the work of Honeycutt (1948) in America that the hair of infants is a more successful bait, and that keratinized substrata buried in soils on the shores of lakes and pools, especially in places where keratin-containing materials naturally occur, are better sources of keratinophilic phycomycetes than similar substrata placed in water.

Fruits proved successful as baits, especially when firm and slightly under-ripe with undamaged waterproof skins, which were pricked in one or two places with a sterile needle. The substrata which produced the greatest number of different fungi, however, were the waterlogged twigs of deciduous trees. It is probable that the crevices and openings on their surfaces provided good anchorage and protection for zoospores. In fact the reproductive branches of many aquatic fungi grew out from the lenticels. Therefore, it was desirable that the water at collection stations should be relatively free from silt in suspension, which tends to fill up these openings. Hence stations where the water was almost still and free from disturbance proved to be the most favourable.

Species recorded and their occurrence

Table 1 lists 44 species recorded during 1955. Twelve were new British records (Perrott, 1959) and many more are not commonly recorded. The list also contains representatives of all the groups of aquatic phycomycetes except the Chytridiales, which were excluded from this study. The occurrence of these species in 108 collections taken monthly from nine stations is also indicated. Presence in a collection is recorded as one occurrence, irrespective of abundance of growths and the number of substrata on which a species occurred.

Each of the four sets of collecting stations, differing in the pH of their waters, is shown to have a fungal population which differs from the others in numbers of species and specific composition. From the three stations with neutrally acid waters a total of 37 species was obtained, in comparison with only 24 species from the three stations with constantly alkaline waters.

Text-fig. 2 shows the percentage occurrence of 15 species at these two groups of collecting stations, the total number of occurrences of a species being expressed as a percentage of the total number of collections. It shows that *Monoblepharis polymorpha* Cornu, *M. insignis* Thaxter, *M. fasciculata* Thaxter, *M. bullata* Perrott, and *M. sphaerica* Cornu, were to be found only in neutrally acid waters. The most commonly occurring species in this investigation was *Apodachlya pyrifera* Zopf, the majority of the records being from constantly alkaline waters. This preference for such waters was also shown by *Phytophthora gonapodyides* (Petersen) Buisman. On the other hand,

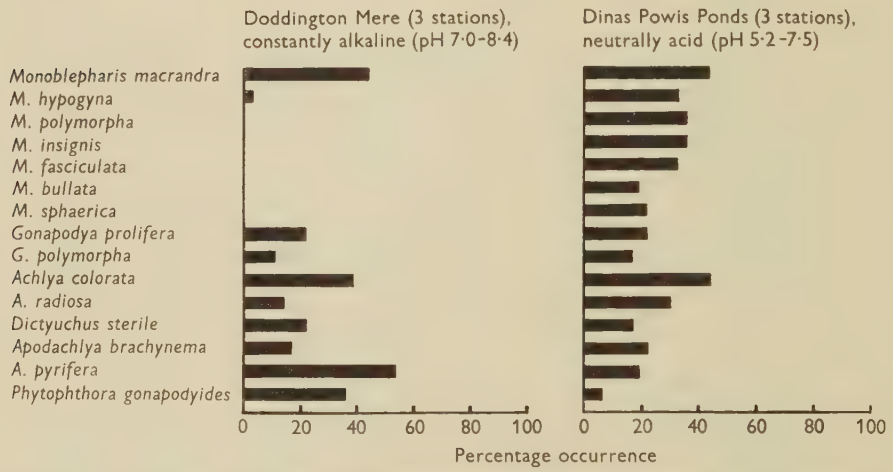
Table 1. *Occurrence of aquatic phycomycetes in* 108 *collections taken from nine collecting stations at monthly intervals for* 1 *year*

Species collected (excluding chytridiales)	Constantly alkaline waters: Doddington Mere (3)	Constantly alkaline waters: Keele Lakes (2)	Neutrally alkaline waters. Bogs Wood Pond (1)	Neutrally acid waters. Dinas Powis Ponds (3)	Total number of occurrences
Blastocladiales					
Blastocladiaceae					
Blastocladia globosa	2	3	1	—	6
Monoblepharidales					
Monoblepharidaceae					
Monoblepharis macrandra	16	6	3	16	41
M. hypogyna	1	—	—	12	13
*M. polymorpha**	—	—	—	13	13
*M. insignis**	—	—	—	13	13
*M. fasciculata**	—	—	—	12	12
*M. bullata**	—	—	—	7	7
M. sphaerica	—	—	—	8	8
Gonapodya prolifera	8	5	2	8	23
G. polymorpha	4	1	2	6	13
Plasmodiophorales					
Plasmodiophoraceae					
Woronina polycystis	3	1	—	—	4
Saprolegniales					
Saprolegniaceae					
Pythiopsis cymosa	1	2	—	—	3
Saprolegnia asterophora	3	3	1	1	8
S. delica	—	1	—	—	1
S. ferax	1	2	—	4	7
S. hypogyna	13	3	1	1	18
S. litoralis	—	—	—	1	1
S. monoica	—	—	—	1	1
Isoachyla monilifera	1	—	—	1	2
Achlya apiculata	1	—	—	1	2
A. colorata	9	6	3	16	34
A. flagellata	—	—	—	2	2
A. imperfecta	1	—	—	3	4
A. racemosa	5	—	2	1	8
A. radiosa	5	2	—	11	18
Aphanomyces stellatus	—	—	—	1	1
A. laevis	—	—	—	1	1
Dictyuchus sterile	5	2	3	5	15
Leptomitales					
Leptomitaceae					
*Apodachlya brachynema**	5	2	—	8	15
*A. minima**	—	—	—	1	1
*A. punctata**	—	—	—	2	2
A. pyrifera	19	10	2	7	38
*A. seriata**	—	1	—	1	2
Rhipidiaceae					
Rhipidium americanum	—	—	—	3	3
R. parthenosporum	—	1	—	2	3
*Mindeniella spinospora**	2	—	—	—	2
*Araiospora pulchra**	—	—	—	1	1
Lagenidiales					
Olpidiopsidaceae					
Olpidiopsis saprolegniae	1	—	—	1	2
*O. vexans**	—	—	—	1	1
Peronosporales					
Pythiaceae					
Phytopthora gonapodyides	13	9	2	2	26
P. syringae	1	—	—	—	1
*Pythium catenulatum**	—	—	—	1	1
P. proliferum	—	1	—	4	5
P. undulatum	1	—	—	—	1

* New British records. — = No record.

Achlya colorata Pringsheim, a very commonly recorded species, together with *Dictyuchus sterile* Coker, occurs almost equally in constantly alkaline and neutrally acid waters.

The distribution of members of the Monoblepharidaceae proved to be interesting in several respects. *M. macrandra* (Lagerheim) Woronin, the most commonly recorded species of the genus, was found in waters ranging from constantly alkaline to neutrally acid. This is possibly why it is one of the species most commonly recorded by other authors. In contrast, the rarely recorded endogenous species *M. fasciculata*, *M. insignis*, *M. sphaerica* and *M. bullata* were found only in neutrally acid waters. It is worthy of note that the only sporadic collection which produced these four species was made at Styal in Cheshire, from a pond the waters of which were also in



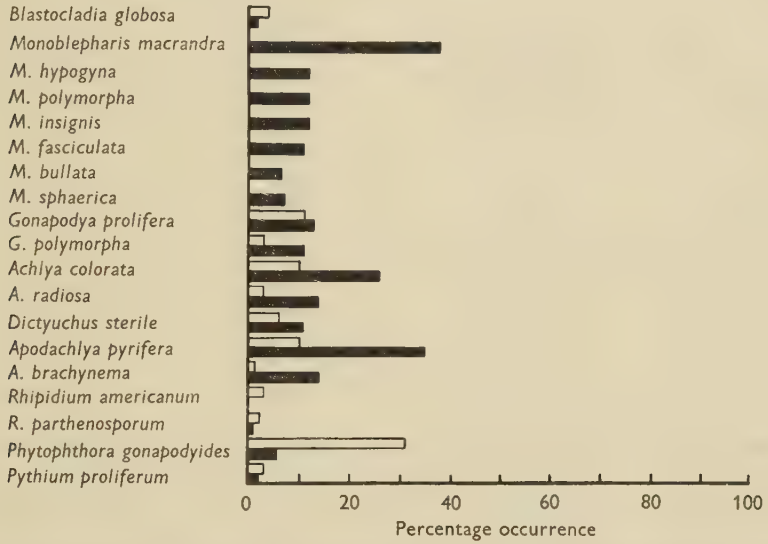
Text-fig. 2. Percentage occurrence of fifteen species of aquatic phycomycetes in constantly alkaline and neutrally acid waters. (Based on 144 collections taken monthly at six collecting stations.)

the neutrally acid range. Both species of *Gonapodya*, however, occurred in waters ranging from constantly alkaline to neutrally acid. The description of sexual reproduction in *Gonapodya* (Johns & Benjamin, 1954) provided convincing evidence of its close relationships with *Monoblepharis* and *Monoblepharella*. Sexual reproduction in *Gonapodya prolifera* (Cornu) Fischer and *G. polymorpha* Thaxter was first seen during this investigation in the autumn of 1954, and as this is the first record from Britain is illustrated in Pl. 1.

Twigs were the baits which produced the greatest number of species. Many were confined to these baits, notably all species of *Monoblepharis*, which occurred on twigs of *Fraxinus excelsior* L., *Ulmus* sp., *Larix decidua* Mill, *Acer pseudoplatanus* L. and *Quercus robur* L. (Text-fig. 3). Three species of *Apodachlya*—*A. minima* Coker & Leitner, *A. punctata* Minden and *A. seriata* Lund—also occurred only on twigs of *Quercus robur*.

A. pyrifera grew on both twigs and fruits, but 48 of its 59 occurrences were on twigs and the remainder on tomatoes, grapes and rosaceous fruits. *Gonapodya prolifera*, on the other hand, had a fairly even distribution

on twigs (14 occurrences) and fruits (12), while *G. polymorpha* was more common on twigs. *Achlya colorata* grew on a wide range of substrata, such as apple, rose hips, hawthorn fruits, tomato and twigs of ash, oak, elm, alder and sycamore, but 28 of 39 occurrences were on twigs. *Phytophthora gonapodyides* was also found on both twigs and fruits, but only six occurrences were on twigs (oak and sycamore) and 31 on rosaceous fruits, tomatoes and grapes. *Rhipidium americanum* Thaxter, *Pythium catenulatum* Matthews and *Phytophthora syringae* (Klebahn) Klebahn occurred only on rose hips, and *Pythium undulatum* Petersen only on apple. *Blastocladia globosa* Kanouse, which has previously been recorded as growing only on fruits,



Text-fig. 3. Percentage occurrence of nineteen species of phycomycetes on two types of substrate, based on 108 collections of each type at substrate taken monthly at nine collecting stations. Type of substrate: ■, twigs; □, fruits.

and particularly hard fruits, such as those of hawthorn, crab-apple and *Cornus officinalis*, was found to occur on tomato fruits and twigs of ash and oak. *Mindeniella spinospora* Kanouse also occurred on both twigs and fruits, though previously recorded only upon rosaceous fruits. This species and *Araiospora pulchra* Thaxter have previously been recorded only from the United States.

Periodicity

When records of occurrence are examined with reference to the time of collection and the appearance of an identifiable growth on the substratum, another probable reason for the infrequent collection of some species becomes clear.

Table 2 summarizes the occurrence of *Monoblepharis insignis* based on records of one collection each month at each group of collecting stations (see Table 1) during 1955 and also on sporadic collections; *M. insignis* was not found at those stations or on those dates which do not appear in

Table 2. The heading 'date of identification' refers to the date of appearance of fungus mycelium bearing sexual organs, which are necessary for identification. The table shows that this species had two periods of germination and growth in 1955, one in March, April and May, and the other in October and November, when vigorous identifiable growths were produced in two to four weeks. On the other hand, twigs collected in summer or winter produced identifiable growths only after 6 weeks to 4 months, the length of time varying according to whether the substratum was collected near the end or near the beginning of the dormant period.

Table 2. *The occurrence of* Monoblepharis insignis *and* M. insignis *var.* minor

Locality (with period of monthly collections—dates inclusive)	Date of field collections	Date of identification
Dinas Powis, pond 15 (Jan.–Dec. 1955)	27 Jan. 1955	26 Mar. 1955
Dinas Powis, pond 9 (Jan.–Dec. 1955)	20 Feb. 1955	8 Apr. 1955
	12 Mar. 1955	9 Apr. 1955
	8 May 1955	20 May 1955
	2 July 1955	4 Oct. 1955
	12 Sept. 1955	4 Oct. 1955
	28 Oct. 1955	12 Nov. 1955
	27 Dec. 1955	17 Mar. 1956
Dinas Powis, pond 5 (Jan.–Dec. 1955)	20 Feb. 1955	8 Apr. 1955
	12 Mar. 1955	9 Apr. 1955
	5 Apr. 1955	22 May 1955
	8 May 1955	22 May 1955
	10 June 1955	14 Oct. 1955
	27 Dec. 1955	17 Mar. 1956
Styal, Cheshire, pond 11 (single collection)	13 Aug. 1954	25 Feb. 1955

These data were confirmed by subsequent collections made during 1956 and 1957, the onset and ending of periods of germination and growth varying slightly according to variations in water temperature in different seasons.

A similar summary for *M. macrandra* (Table 3) also shows a period of germination and growth in March, April and May, and another in October and November, but its tolerance of habitats with a wide pH range no doubt accounts for its more frequent collection.

It appears therefore that the oospores of *M. macrandra* remain dormant during the winter and do not germinate until March, April or May. Very shortly after germination sexual organs are produced on the newly formed mycelia followed almost immediately by oospore production. These spring-formed oospores remain dormant until October or November, when a fresh crop of sex organs and oospores is produced It is these autumn-formed oospores which germinate in the following spring. Hence in any one year there are two periods of germination and growth, one in spring and another in autumn, with dormant periods in summer and winter. The autumn period of growth does not occur in every case, and occasionally substrata collected during the summer do not produce identifiable growths until the following spring (see Styal collection, Tables 2 and 3).

A summary of the occurrence during one year of this investigation of nine species of the Monoblepharidaceae and six species of aquatic phycomycetes representative of other groups is given in Text-fig. 4.

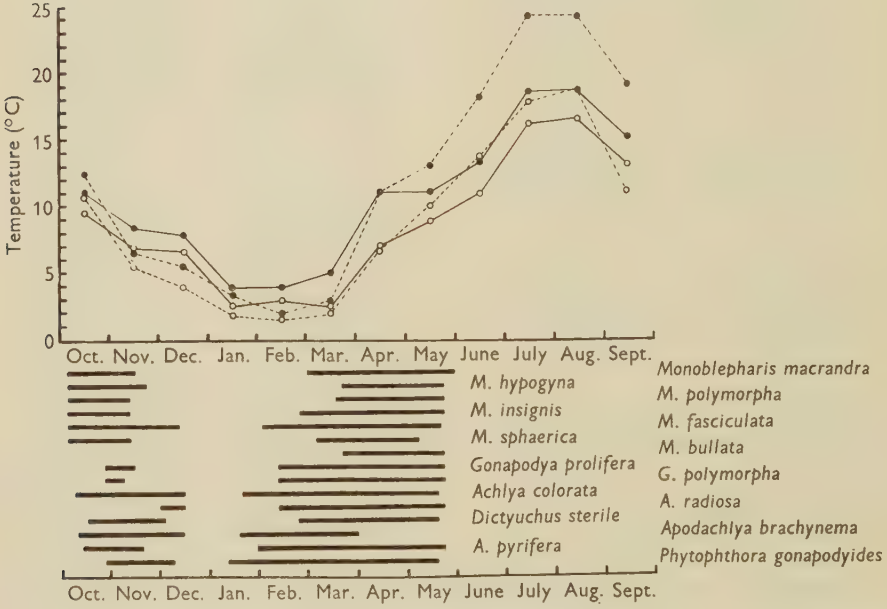
The growth pattern of all species of *Monoblepharis* is similar to that of *M. macrandra*, excepting *M. fasciculata* which shows a longer period of germination and growth, commencing earlier in spring and ending later in autumn, and *M. bullata* which has not been found to germinate in autumn. Both species of *Gonapodya* show a period of growth and germina-

Table 3. *The occurrence of* Monoblepharis macrandra *and* M. macrandra *var.* laevis

Locality (with period of monthly collections—dates inclusive)	Date of field collections	Date of identification (appearance of vigorous growth with sex. rep.)
Doddington Mere Peninsula, north side (Jan.–Dec. 1955)	13 Apr. 1955	8 May 1955
	12 May 1955	30 Mar. 1956
	10 June 1955	10 Oct. 1955
	26 July 1955	16 Oct. 1955
	27 Sept. 1955	28 Oct. 1955
	27 Oct. 1955	5 Apr. 1956
	19 Nov. 1955	6 Apr. 1956
Doddington Mere Peninsula, south side (Jan.–Dec. 1955)	28 Jan. 1955	11 Mar. 1955
	10 Mar. 1955	29 May 1955
	12 May 1955	1 Mar. 1956
	26 July 1955	16 Oct. 1955
	27 Sept. 1955	15 Nov. 1955
	27 Oct. 1955	20 Mar. 1955
Doddington Mere South Bank (Jan.–Dec. 1955)	26 July 1955	29 Mar. 1956
	27 Sept. 1955	27 Oct. 1956
	27 Oct. 1955	20 Mar. 1955
Keele Lake I (Jan.–Dec. 1955)	4 Apr. 1955	4 May 1955
	4 June 1955	4 Nov. 1955
	20 Dec. 1955	7 Mar. 1955
Keele Lake II (Jan.–Dec. 1955)	4 Mar. 1955	20 Apr. 1955
	4 Apr. 1955	18 May 1955
	14 May 1955	29 Mar. 1956
Bogs Wood Ponds (Jan.–Dec. 1955)	12 Mar. 1955	4 Apr. 1955
	14 Apr. 1955	4 May 1955
	14 July 1955	4 Mar. 1956
Dinas Powis, pond 15 (Jan.–Dec. 1955)	27 Jan. 1955	26 Mar. 1955
	20 Feb. 1955	8 Apr. 1955
	12 Mar. 1955	9 Apr. 1955
	5 Apr. 1955	7 May 1955
	28 Oct. 1955	15 Mar. 1956
Dinas Powis, pond 9 (Jan.–Dec. 1955)	20 Feb. 1955	8 Apr. 1955
	12 Mar. 1955	1 Apr. 1955
	8 May 1955	20 May 1955
	10 June 1955	4 Oct. 1955
	2 July 1955	4 Oct. 1955
	12 Sept. 1955	8 Oct. 1955
	28 Oct. 1955	12 Nov. 1955
Dinas Powis, pond 5 (Jan.–Dec. 1955)	20 Feb. 1955	18 Apr. 1955
	12 Mar. 1955	9 Apr. 1955
	8 May 1955	22 May 1955
	10 June 1955	14 Oct. 1955
Ullswater (single collection)	28 May 1954	11 Mar. 1955
Blelham Tarn (single collection)	29 May 1954	18 May 1955
Pond I, Styal, Cheshire	13 Aug. 1954	1 Apr. 1955
Pond II, Styal, Cheshire	13 Aug. 1954	25 Feb. 1955
Blaise Castle, Bristol (pond)	10 Sept. 1955	30 Mar. 1956

tion similar to that in *Monoblepharis* during the spring, but the autumn period is much shorter and commences later.

From Text-fig. 4 it can readily be seen that the spring germination period for members of the Monoblepharidaceae follows low temperatures (which included freezing), while the autumn germination period follows the highest temperatures, for the growth periods range from 3° to 15° C., with a range of 7°–11° C. for the majority of the time. In this connexion it is interesting to note that although natural growths of *Monoblepharis* with sexual organs could be induced at 3° C., asexual reproductive organs were rarely formed at this temperature, and then only in the exogenous



Text-fig. 4. Maximum and minimum water temperatures (monthly means) at two collecting stations from October 1954 to September 1955 and seasonal occurrence of fifteen species of aquatic phycomycetes. Water temperatures (monthly means): maximum Keele Lake, ●—●; Doddington Lake, ●– – –●; minimum Keele Lake, ○—○; Doddington Lake, ○– – –○.

species. In fact zoosporangia in the endogenous species *M. insignis*, *M. fasciculata* and *M. bullata* were unknown until methods of keeping substrates at 10° and 15° C. were devised. In pure culture the optimum temperature for sporangial production and dehiscence proved to be 15° C. (Perrott, 1958). It seems likely therefore that water temperatures play an important part in inducing asexual reproduction and vigorous growth. But temperature is not the only factor involved, for even though suitable temperatures are provided in the laboratory for oospores collected during the summer or winter period of dormancy, germination does not take place until October or March respectively.

Summaries of occurrence of species from other orders of the aquatic

phycomycetes also show a similar seasonal periodicity. *Apodachlya pyrifera* (Text-fig. 4), for example, shows a spring period of germination and growth, but including February as well as March, April and May, while the autumn growth period does not begin until mid-October. *Achlya colorata* has a pattern of growth similar to *Apodachlya pyrifera*, but the autumn growth period often extends into the beginning of December and the spring period includes the end of January, so that the only marked period of dormancy for this species is during the summer. This is also the case for *Phytophthora gonapodyides*, where the winter period of dormancy is even less marked and autumn growth does not begin until near the end of October. This corresponds with the findings of Waterhouse (1942) for collections of this species from river water. Furthermore, it is interesting to note that Hickman (1940) has shown that *P. fragariae* infects strawberry roots from the soil only from autumn until spring, and that it cannot be isolated at other times of the year. *P. ilicis* also has been shown to behave similarly (Buddenhagen & Young, 1957), attacking leaves and twigs of English holly during winter and spring, during which period it is readily isolated, although for the rest of the year it appears to be inactive.

Discussion

From the observations made during this investigation it appears that many species of aquatic phycomycetes show a seasonal periodicity of growth in spring and autumn, important factors in germination and subsequent growth being temperature and the maturation of the oospore. It also seems probable that the supposed rarity of many aquatic phycomycetes is due to lack of knowledge of the most suitable time for collection, the type of habitat and substratum favoured, and a technique for subsequent treatment.

By the use of the methods described here, species of *Monoblepharis* have been obtained from all stations where regular collections were made (Table 3) and from many sporadic collections (Loch Lomond, Lydney). These and published records from East Anglia and south-east England provide evidence of a relatively wide distribution for this supposedly rare genus.

My sincere thanks are due to Prof. A. R. Gemmell and Prof. C. W. Wardlaw for their interest in this work and to the Royal Society for a research grant. I am indebted to Mr E. Ashby for the preparation of the photomicrographs.

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EXPLANATION OF PLATE 1

Gonapodya prolifera

Fig. 1. Branched hyphae bearing proliferated male gametangia at their tips. Two gametangia are discharging their antherozoids. Each antherozoid has a conspicuous anterior cluster of refractive granules. × 500.

Fig. 2. Mature female gametangia containing amoeboid oospheres and thin-walled oospores. One empty gametangium shows proliferation. × 500.

(*Accepted for publication* 7 *March* 1959)

(*Facing p.* 30)

Trans. Brit. mycol. Soc. **43** (1), 31–50 (1960).

OCCURRENCE OF *ITERSONILIA* AND *TILLETIOPSIS* ON LESIONS CAUSED BY *ENTYLOMA*

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(With 5 Text-figures)

Itersonilia perplexans is prevalent on lesions caused by species of *Entyloma* and by other plant pathogenic fungi and is occasionally present on 'healthy' leaves. *Tilletiopsis washingtonensis* and *T. minor* are present on most leaves whether or not attacked by *Entyloma*.

Chlamydospores of *E. calendulae* and *E. dahliae* germinate in the host forming needle-shaped and allantoid sporidia; the latter superficially resemble the ballistospores of *T. washingtonensis* but are distinguishable from them when they germinate. Inoculations demonstrated that symptoms characteristic of infection by *Entyloma* are produced by the needle-shaped sporidia; no connexion was established between the life cycles of *Entyloma* and either *I. perplexans* or *T. washingtonensis*.

Species of *Entyloma* produce lesions in the leaves of their hosts where the mycelium eventually becomes transformed into a mass of thick-walled, hyaline chlamydospores. Frequently, reports have appeared of exogenous thin-walled spores on the surface of the lesions, described variously as sporidia derived from the germinated chlamydospores or as mycelial conidia. The role of these thin-walled spores in the life-cycles of the smuts is uncertain. 'Half-moon-shaped sporidia' described by Stempell (1935) from lesions caused by *E. calendulae* (Oudem.) de Bary have been stated by Derx (1948) and Olive (1952) to represent *Itersonilia perplexans* Derx. The accounts of Derx (1930), Nyland (1950) and Tubaki (1952) of the widespread occurrence of *Tilletiopsis* spp. on the leaves of 'healthy' plants suggest that members of this genus may likewise have been assigned to *Entyloma*.

Living material of several species of *Entyloma* was therefore examined for thin-walled spores of this kind associated with the lesions, in order to determine whether or not any of them belong to the *Entyloma*. In order to facilitate description, all thin-walled spores isolated from the surface of lesions caused by *Entyloma* and from the germinated chlamydospores, and all similar thin-walled spores formed by isolates in culture are here called 'sporidia'.

Materials and methods

As a greater range of material could be procured in France, where a high proportion of the European species has been recorded and the commoner ones are more readily available, ten species were examined from various

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French localities and only 3 from England. Of these, *E. calendulae* on cultivars of *Calendula officinalis* L., and *E. dahliae* Sydow on cultivars of dahlia were studied in greater detail.

Collection and culture of material

Leaves infected by *Entyloma* were sampled for surface spores as soon as possible after collection. Many of these spores are forcibly discharged by the drop-excretion mechanism (ballistospores) and were easily obtained in culture by fixing pieces of leaf, previously washed and dried, inside the lids of Petri dishes with strips of moistened cellophane. The resulting spore deposit was collected either on a suitable agar medium in the lower half of the dish or on slides treated with Mayer's slide fixative for later cytological investigation. Other surface spores which were not forcibly discharged were collected by scraping the leaf surface gently with a sterile glass rod and dipping this into a small drop of distilled water which was then spread over agar. For monospore cultures single spores were picked up from the surface of the medium on the end of a dry, finely drawn-out glass rod and transferred to a marked area on fresh agar.

It is difficult to obtain uncontaminated chlamydospore material from mature lesions because the spores have to be teased out of a leaf which is already heavily invaded by secondary infections of sporobolomycetes and other fungi and bacteria. It was found most effective to grind washed pieces of infected leaf in a mortar with dilute mercuric chloride solution and to separate the spore suspension from the leaf material by centrifugation. The spores were then washed several times with sterile distilled water. Hanging drops of the spore suspension were kept at 24° C. in the light in moist Petri dishes. After allowing time for germination and growth, the spores were transferred to an agar surface. It was not practicable to initiate germination directly on the agar surface because the aerobic bacteria, which remain after even the most careful washing of spores, overgrow the promycelia before they can develop far. In hanging drops, however, the promycelia get ahead before they are transferred to agar where their further development can easily be followed under the microscope.

All measurements of thin-walled spores and mycelium were made from material recently isolated and spread on the surface of agar and covered by a thin coverslip.

A 2% solution of malt extract, solidified with 2·5% agar, was the standard medium, but most of the fungi grew equally well on Sabouraud's glucose, potato-extract and potato-dextrose agars.

Cytological technique

For preferential staining of the mycelium in the host, pieces of the infected leaf were cleared in cold chloral hydrate solution (McBride, 1936) and stained in acid fuchsin. Alternatively, a commercial preparation of pectinase was used to separate the cells of the host and display the parasitic mycelium (Fraymouth & Hawker, 1952). Microtomed sections were made of fixed leaf material embedded in paraffin wax.

Two methods satisfactory for staining the nuclei were: (1) a modification of Heidenhain's iron alum haematoxylin technique, material being fixed in Sansome's modification of Navaschin's fluid for 24 hr. or more and stained in 0·5 % aqueous haematoxylin, using 3 % ferric chloride before and after staining; and (2) Olive's propiono-carmine method (Olive, 1952), used on slide smears fixed for ½ hr. in a modified Carnoy's fluid (glacial acetic acid 1, absolute alcohol 2, chloroform 3); the slides were then passed down an alcohol series to water and mordanted in 4 % ferric alum solution before staining in 2 % carmine in 40 % propionic acid. In both methods the stained slides were washed, dehydrated and mounted in Euparal. Propiono-carmine was used for staining all stages except the chlamydospores, where penetration of the Carnoy's fixative through the thick walls was not sufficient, method (1) being more satisfactory.

Results

'*Sporidia*' *from lesions of* Entyloma calendulae *on* Calendula officinalis

The several different kinds of spore obtainable from the surface of lesions of *E. calendulae* were examined before attempting to determine how many of these were actually part of the life-cycle of the smut. Four kinds of spore were repeatedly isolated:

Allantoid '*sporidia*', 11–24 (av. 15·5) × 2–3·5 μ; thin-walled, hyaline, slightly curved and more pointed at the attachment end; forcibly discharged from both surfaces of the leaf; uninucleate; of two kinds (*a*) and (*b*) distinguished by their mode of germination:

(*a*) These germinate in water and on an agar surface at both ends to give a fine mycelium 1–2 μ wide, rarely branching, and with very few septa. The mycelium follows a twisting course on the surface of the medium with limited branching, giving rise to a sparse mycelium which slowly extends over the surface. No buds are formed, but allantoid 'sporidia' like those discharged from the leaf are produced on the surface of the colonies at the ends of short side branches and discharged by the drop-excretion mechanism. Occasionally, very long, narrow, needle-shaped 'sporidia' are formed from similar side branches and project into the air above the colony; these, very few of which were measured, are about 20–40 × 2 μ and are not forcibly discharged.

(*b*) These also germinate in water and on an agar surface at both ends to give a fine, branched mycelium, 1–2 μ wide; septa are rare but more common than in (*a*). The twisting of the mycelium so characteristic of (*a*) 'sporidia' does not occur, and the mycelium grows more quickly over the surface of the medium. Germination of the (*b*) 'sporidium' is often followed by the formation of many yeast-like buds. Allantoid spores like those isolated in the spore print from the smut lesion and like those on (*a*) 'sporidia' mycelia are later produced at the surface of the colonies on the ends of short side branches and discharged by drop-excretion mechanism. Hyaline chlamydospores, 12–14 μ diam., are formed in old cultures.

Many spore prints were examined cytologically and all 'sporidia' were uninucleate. Similar prints examined for germination included representatives of both kinds of 'sporidium'. It is likely, therefore, though not absolutely certain, that both types have a single nucleus. As it was difficult to measure both kinds, the measurements of the allantoid 'sporidia' given above represent the range of 'sporidium' size found in a lesion print, and thus cover both kinds.

'*Half-moon-shaped sporidia*', 13–21 (av. 16) × 7–15 (av. 11) μ, thin-walled, hyaline; shape approximately that of a $\frac{1}{6}$ segment of a sphere, the 'ends' being rounded; forcibly discharged from both leaf surfaces; binucleate.

These germinate in water and on an agar surface by one, rarely two, germ-tubes which arise from the curved surface of the spore and form a hypha, 3–5 μ wide, which becomes septate with simple cross-walls. Later septa are formed only in association with clamp connexions which arise at intervals, a side branch often arising later from the clamp branch, or opposite it. In young cultures of several hours 'half-moon-shaped sporidia' similar to the 'sporidia' isolated from the smut lesion are formed at the tips of tapering side branches and discharged by drop-excretion mechanism. In cultures of a week or more chlamydospores 10–15 (av. 13) μ diam. are formed on the ageing parts of the mycelium. They are spherical, occasionally obpyriform, with rather thinner walls and less dense contents than *Entyloma* chlamydospores formed in the leaves.

When 'half-moon-shaped sporidia' germinate in contact with a hard surface such as glass or the leaf epidermis, the germ-tube first swells to give a lobed appressorium-like structure with dense contents, from which one or more hyphae grow out to form a typical clamp mycelium.

Needle-shaped '*sporidia*', 27–57 (av. 37) × 1–2 μ; thin-walled, hyaline, usually straight, but sometimes slightly curved, tapering at the distal end; discharged by dropping from the parent hypha; binucleate.

Germination takes place in water and on an agar surface at both ends to give a hypha 1–2 μ wide, which forms a rarely septate, sparsely branched mycelium like that formed by the allantoid (*a*) 'sporidia'. Similarly also, the hyphae follow a twisting course on the agar surface. Needle-shaped 'sporidia' are abstricted from the ends of short side branches and allantoid 'sporidia' are formed on short sterigmata and are discharged by drop-excretion mechanism.

Proportion and order of '*sporidium*' *production.* The examination of several hundred lesions of *E. calendulae* throughout five seasons showed that 'sporidium' production was sporadic, probably owing to weather conditions. While many were formed after dull weather or rain, few were produced after hot weather and drought. The age of the lesion appeared to have some influence on production, older ones often having a larger crop than younger. Because of the early production of chlamydospores it was difficult to determine whether any 'sporidia' appeared before the chlamydospores were formed. Allantoid (*b*) 'sporidia' were, however, occasionally found on very young lesions in which there were only very immature chlamydospores. Needle-shaped 'sporidia' were found on most lesions with mature chlamydospores, but were not found in very dry

conditions. If a lesion collected on a dry day was put in a refrigerator at 0–5° C. for a few hours, needle-shaped 'sporidia' would sometimes develop when the leaf was returned to room temperature.

Whenever a leaf infected by *E. calendulae* was suspended over agar for making a print, note was taken of the order in which these 'sporidia' were discharged and the position of origin. In general, allantoid (*a*) 'sporidia' fell first, followed by (*b*) or 'half-moon-shaped' ones. Allantoid (*a*) 'sporidia' ceased to appear after about 24 hr. but the (*b*) and 'half-moon-shaped' ones would continue to be discharged for several days, the latter even throughout the course of a week. All three kinds were obtained from the surface of some lesions, but other lesions only provided one or two. Where two or more kinds were discharged from a single lesion it was more usual for the different kinds to fall from distinct areas of the lesion than from the same areas.

The spore print of an entire leaf showed that allantoid (*a*) 'sporidia' were discharged only from the region of the lesions. 'Half-moon-shaped sporidia' were nearly always confined to the lesions; in two leaves only out of many hundreds were they found on that part of the agar under the non-lesion part of the leaf. Allantoid (*b*) 'sporidia', however, were discharged from all parts of the leaf and were not localized under the lesions. Similarly, spores of *Sporobolomyces* spp. and very small colourless allantoid spores which formed yeast-like colonies and probably belonged to *Tilletiopsis* spp. were also secured from the non-lesion part of the leaf surface. Both of these latter were also found beneath lesions, but when the 'half-moon-shaped' and allantoid 'sporidia' were being discharged the *Sporobolomyces* spores and the very small ballistospores were not usually present.

The chlamydospores of Entyloma calendulae

Very young chlamydospores have two nuclei that stain with propiono-carmine. In mature chlamydospores a single very large body stains deeply with Heidenhain's haematoxylin, but it is uncertain whether this is the nucleus or a storage product. Germination was studied in chlamydospores teased from mature lesions and kept in hanging drops which were later transferred to agar; the nuclei were examined in propiono-carmine smears (Fig. 1).

Germination is first evident when the wall cracks and the promycelium emerges. Sometimes this begins while the chlamydospore is still in the host tissues, at other times the germ-tube may emerge during the 24 hr. after the hanging drop is set up. The promycelium widens almost at once, remaining constricted at the point where it leaves the chlamydospore. Elongation is extremely rapid at this stage; most preparations show four, presumably haploid, nuclei, but occasionally a single undivided nucleus is seen at the base of a young promycelium. This nucleus is presumably the one that is stainable with haematoxylin in the mature chlamydospore and, by analogy with other smuts, is assumed to be diploid. The four or more nuclei that result from the divisions of the original single nucleus are arranged in a line near the base of the promycelium. Meanwhile, some very small protuberances appear at the tip and elongate rapidly to form

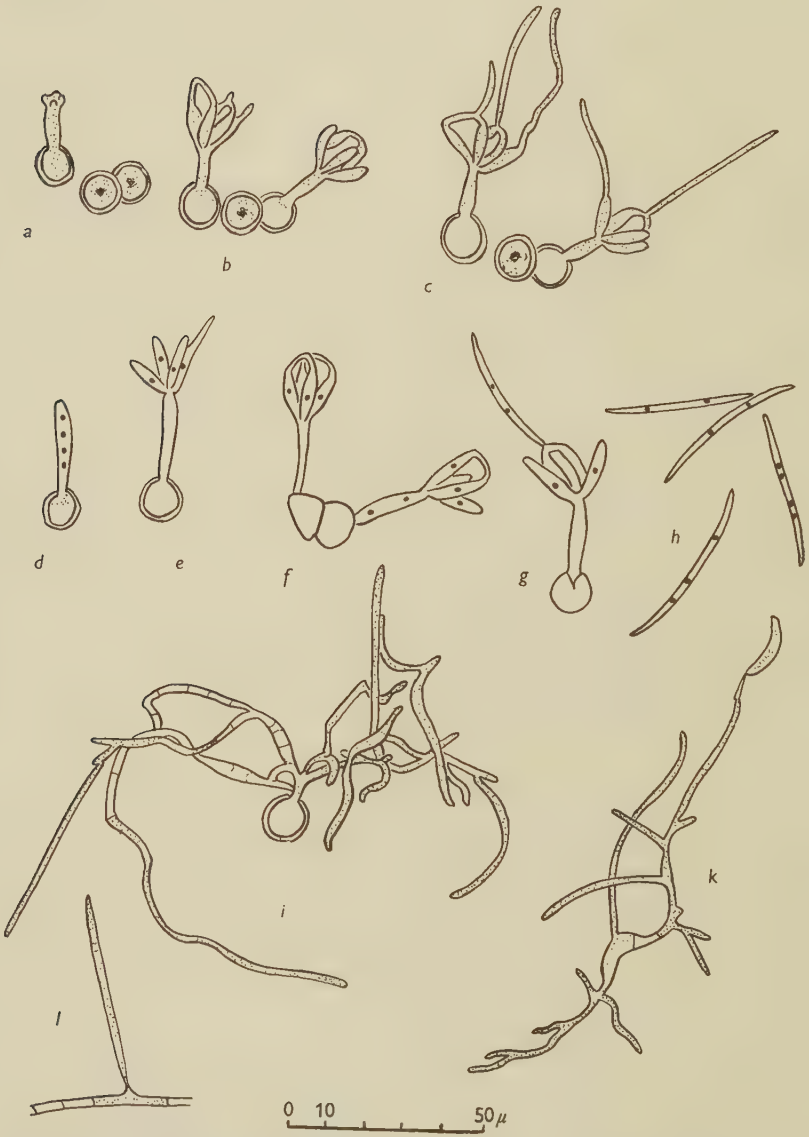

Fig. 1. *Entyloma calendulae*. Stages in the germination of the chlamydospores. *a*, Young promycelium; *b*, conjugation between branches of the promycelium; *c*, formation of needle-shaped sporidia, and the outgrowth of one of the unfused branches; *d–g*, stages in germination from drawings of propiono-carmine preparations, showing the nuclei; *h*, needle-shaped sporidia from propiono-carmine preparations, showing binucleate sporidia and sporidia in which the nuclei have divided; *i*, young mycelial colony formed on agar by a germinating chlamydospore, showing a needle-shaped sporidium forming on the left; *k*, allantoid sporidium from a colony formed by a chlamydospore (a second allantoid sporidium is being formed on the right on a sterigma); *l*, part of the same colony as in *k*, showing a needle-shaped sporidium.

four or more, often up to eight, promycelial branches. Not until these branches are well formed do the nuclei move to positions near the bases of the branches, into each one of which a nucleus passes at about the time the branches reach their maximum length. All the contents of the chlamydospore meanwhile pass into the promycelium and accumulate at its tip. Vacuolation occurs at the base, giving the appearance of many cross-walls; in fact one or occasionally two cross-walls are formed, cutting off the empty spore from the developing promycelium. The size of the promycelium varies greatly with the conditions in the drop, being 8–40 μ long, 2–8 μ diam. The promycelial branches are straight, 10–20 × 2–3 μ, and in liquid medium they stand out at a wide angle in a crown at the tip of the promycelium; on solid medium they often diverge less from one another. Each branch has a single large nucleus which takes up a position near the centre of the branch.

Conjugation of the branches in pairs occurs mainly at the tip, occasionally at the base, and is often brought about by means of a long conjugation tube, though sometimes by a shorter one. Stages in the formation of this tube are difficult to trace, but occasionally two unfused branches can be seen, each with a distal extension, and it is probable that fusion occurs between the tips of two such protuberances, and not between a single prolongation of one branch with the tip of an unextended branch. Alternatively, the two extensions seen in these few instances may have been outgrowing branches destined never to fuse, as happens when an odd number of branches occurs in a crown.

On the distal part of the promycelium a hump appears into which the protoplasm with the two nuclei from the conjugated promycelial branches passes. This hump extends to form a slender, straight, or sometimes slightly curved, binucleate sporidium, pointed at its tip, 27–57 × 1–2 μ. These needle-shaped sporidia are easily detached, but in a hanging drop transferred to agar often three or four remain attached to a germinated chlamydospore and can be picked off individually with a glass rod (Fig. 1 *h*).

Unconjugated promycelial branches often grow out at the tip to form a mycelium. Needle-shaped sporidia were, however, never found connected with unfused promycelial branches, nor were uninucleate needle-shaped sporidia, like the occasional ones reported by Kaiser (1936), ever seen. In detached sporidia there are often three or four nuclei, presumably formed by the division of one or both of the two nuclei of the sporidium, and the two products of one division remain close to one another in the early stages of germination.

Sporidia germinate well on the surface of agar, usually at both ends, to give a narrow, sparsely branched mycelium, 1·5–2 μ wide. This mycelium has few cross-walls, and grows in a markedly 'twisting' manner. The occasional side branches often grow out above the medium and produce needle-shaped sporidia very like the parent sporidium; they are easily detached but not forcibly discharged. Occasionally also, allantoid sporidia are formed on short side branches and are forcibly discharged by drop-excretion (Fig. 1 *i*, *k*, *l*).

Thus, when the chlamydospores of *E. calendulae* germinate only two kinds of sporidium are formed, allantoid (*a*) and needle-shaped ones

morphologically like the needle-shaped spores isolated by scraping the lesion surface and germinating to form the same kind of mycelium. This suggests that the foliar needle-shaped 'sporidia' are formed as a result of the germination of chlamydospores in the host, and that some of the allantoid (*a*) 'sporidia' may also arise from germinating chlamydospores in the host. It is possible, however, that some leaf 'sporidia' are produced directly by the parasitic mycelium, as would appear to be the way in which such 'sporidia' are produced by those species of *Entyloma* where the chlamydospores do not germinate *in situ*.

Chlamydospores never produced 'half-moon-shaped' or allantoid (*b*) 'sporidia'.

The parasitic mycelium of Entyloma calendulae

If a leaf surface discharging 'sporidia' is examined microscopically, the 'frosty' appearance of the surface is seen to be partly caused by tufts of hyphae protruding from the stomata and bearing 'sporidia'. In older lesions a mat of mycelium is formed by the 'sporidia' which have germinated on the surface, and is contributed to by secondary invaders growing on the leaf surface. The frosty effect is increased where tufts of sporidiophores force the guard cells apart and air is admitted to the intercellular spaces of the mesophyll.

The parasitic mycelium of *E. calendulae* and of *E. dahliae*, mounted fresh in water, is 1·3–2 μ diam. A picture of the extent of the parasitic mycelium in three dimensions is obtainable in the following way.

An entire small leaf, or part of a larger one, is cleared in chloral hydrate and the mycelium stained with acid fuchsin. This reveals the horizontal extent of the mycelium. The vertical system is best seen in vertical sections, though the hyphae of this system follow a winding route and tend to be so crowded in the intercellular spaces that it is impossible to follow the course of individual hyphae. Finally, pieces of intact mycelium may be extracted from the host tissues with the aid of 'Pectozyme'.

The limit of the lesion is best seen by viewing a cleared and stained piece of leaf, mounted with the lower surface uppermost on a slide, under an oil-immersion lens. The hyphae radiate in remarkably straight lines from the point of infection and run parallel to the leaf surface between the lower epidermis and the mesophyll. The cross-walls of clamp connexions show up white against the purple-stained contents of the hyphae at infrequent intervals.

The side branches of the horizontal hyphae, which diverge from the parent hypha at an acute angle, often, but not always, arise in association with a clamp connexion. They penetrate between the cells of the lower epidermis and those of the mesophyll and follow a spiral growth between the cells in a direction at right angles to the parent hyphae.

Young chlamydospores arise at the ends of short side branches, and in the extreme centre of even a young lesion a few immature chlamydospores can be found. In older lesions all intercellular spaces are filled with chlamydospores, the mature ones occurring towards the centre of the lesion.

In vertical section the intercellular spaces of the lower mesophyll are seen to be filled with a twisted mass of hyphae, and twisted strands run up

between the cells of the palisade tissue. Hyphae fill the substomatal chambers of both leaf surfaces and pass out to the exterior. The mass of hyphae present in the intercellular spaces makes it very difficult to trace the origin of the sporidiophores. In vertical leaf sections 'chains' of chlamydospores are often evident between the cells of the palisade. This appearance is caused by the crowding together of hyphae, each bearing one or more chlamydospores, as they pass along the intercellular space.

Clamp connexions occur in both young, marginal mycelium and the older, central part of the lesion; they are most clearly demonstrated in mycelium isolated from the host by 'Pectozyme' action on thick hand sections of the leaf (Fig. 2). The pectinase dissolves the middle lamella and

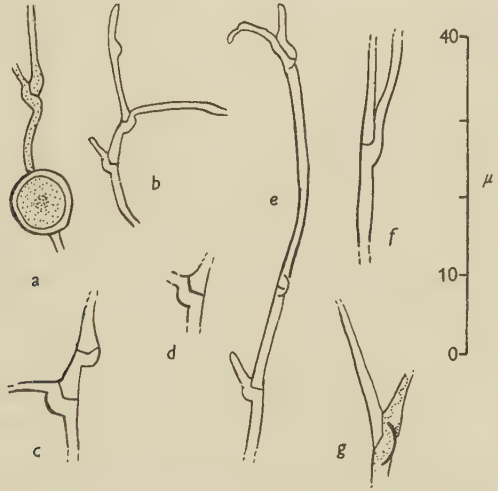

Fig. 2. Parasitic mycelium of *Entyloma calendulae* and *E. dahliae*. *a–d*, *E. dahliae*, showing clamp connexions and a young chlamydospore; *e–g*, *E. calendulae* showing clamp connexions.

the cells can easily be separated by mounting the section under a coverslip and tapping it gently. The ease with which the mycelium separates from the host cells indicates the absence of haustoria.

The sporidia borne by the tufts of parasitic mycelium at the surface of the leaf are very easily knocked off in preparations but young ones sometimes remain attached. In the material examined both allantoid and 'needle-shaped' were seen, but it was very difficult to detect whether they were formed directly on the parasitic mycelium, or indirectly as a result of chlamydospore germination. Sometimes a sporidiophore was followed to its origin from a germinating chlamydospore; usually, however, the mycelium was too tangled to be traceable. A 'half-moon-shaped sporidium' was never found attached to *Entyloma* mycelium issuing from the host plant.

The behaviour of the 'sporidia' in relation to the host

Symptoms characteristic of an infection by *Entyloma* have been induced in a healthy plant by spraying with an aqueous suspension of chlamydospores obtained from a leaf lesion caused by the same species of *Entyloma*.

This has been done with *E. calendulae* on *Calendula officinalis* and *E. dahliae* on *Dahlia* cultivars. Within 3 weeks to 1 month of spraying, many typical lesions appeared, with a pale halo surrounding a region containing young chlamydospores which were later isolated and shown to germinate in the manner typical of the species. Control plants sprayed with water only did not develop these symptoms. It is probable that the greater part of this infection was brought about by the germinating chlamydospores, though the suspension may also have included some foliar 'sporidia', and it is possible that such 'sporidia' alone may infect the host in the absence of chlamydospores. Inoculations were made to test this.

'Sporidia' isolated from the surface of lesions of *E. dahliae* were of the same four kinds found on lesions of *E. calendulae*, and as *Dahlia* plants were more easy to handle for purposes of inoculation most experiments were made with *E. dahliae*.

(A) *Attempts to obtain fusion between allantoid 'sporidia'*. Both 'half-moon-shaped' and needle-shaped 'sporidia' are binucleate but the allantoid (*a*) and (*b*) 'sporidia' are uninucleate. Some kind of fusion might be expected to occur between two uninucleate 'sporidia' before invasion of the host could take place. Monospore cultures of allantoid (*a*) and (*b*) 'sporidia' of the same and different kinds were grown together on agar media containing host decoction, malt extract, or potato extract of various strengths, or buffered media over a range of pH. Spore prints of freshly discharged 'sporidia' from several lesions were also mixed on various media. The only fusions observed in the large number of prints examined were of a few allantoid (*a*) 'sporidia' freshly fallen from lesions of *E. dahliae*, when, on very dilute (0·5 % and 1 %) malt-extract agar, fusions were detected by keeping a 'sporidial' print under observation for several days; tips of branches were seen to approach each other and fuse. Fusions occurred, however, as often between branches from the same 'sporidium' as between those from different 'sporidia'; the mycelium formed after a fusion was the same in appearance as the branches which took part in it. Since, on the dilute media used, the mycelium was exhibiting starvation growth, it seems probable that these fusions were unnatural occurrences induced by the lack of nutriment, though such conditions would be found on the leaf surface where fusion, if it takes place at all, might be expected to occur. 'Sporidia' which had been allowed to fall on the epidermis of a healthy leaf occasionally exhibited similar apparent fusions. As the epidermis had to be stripped off or the leaf cleared in order to see these 'fusions', there was no means of knowing whether they actually were fusions between mycelial branches coming from two different 'sporidia', or between branches from the same 'sporidium', as occasionally happens in artificial culture.

(B) *Inoculation experiments*. The majority of experiments was made with 'sporidia' isolated from *E. dahliae* on to seedlings of Carters' Coltness Gem dahlias. In a few instances 'sporidia' isolated from the lesions of *E. calendulae* were inoculated on to seedlings of Carters' Radio *Calendula*.

Four kinds of 'sporidium' were used: allantoid (*a*), 108 experiments; allantoid (*b*), 52; 'half-moon-shaped', 108; and needle-shaped, 36. The needle-shaped 'sporidia' were either scraped from the lesion surface or

removed directly from germinating chlamydospores. All the other kinds of 'sporidium' were taken from spore prints of leaf lesions. Monospore cultures of all four kinds were maintained, and the methods of inoculation tried were: (1) mass inoculation of the 'sporidia' from an actively growing, recently isolated culture on agar; (2) single 'sporidia' were taken directly from a lesion deposit or from a germinating chlamydospore and transferred immediately to the leaf surface by means of a micromanipulating needle.

The methods used for applying the inoculum to the plant simulated what are probably the major means of spread of the fungus in natural dispersal by air currents and by rain splash. Some 'sporidia' were allowed to shoot on to the leaf, some were placed there dry or were applied in aqueous suspension. In all tests control plants were treated as the inoculated ones, except that water only was placed on the leaves instead of inoculum.

The only occasions when symptoms associated with an infection by *E. dahliae* resulted were when single needle-shaped sporidia, isolated directly from the ends of conjugated promycelial branches of germinating chlamydospores of *E. dahliae*, were placed on the upper surfaces of *Dahlia* leaves. Twelve such inoculations were made and, in three, lesions typical of *E. dahliae* developed and were visible 1 month after inoculation. Chlamydospores were isolated from these lesions and were shown to germinate in the manner typical of spores of *E. dahliae*.

The role of the leaf-surface 'sporidia' in relation to the life cycle of the Entyloma

Of the four kinds of 'sporidium' found associated with lesions of *E. calendulae* and *E. dahliae* only the needle-shaped and the allantoid (*a*) sporidia have proved to be actually part of their life cycle; both are formed when the chlamydospores germinate. The needle-shaped sporidia of *E. dahliae* are capable of reinfecting the host. 'Half-moon-shaped' and allantoid (*b*) 'sporidia' have not been found when the chlamydospore germinates nor in connexion with the vegetative parasitic mycelium. It is probable that they are not connected with the smut at all and are only present on the lesions as contaminants. In order to test this, lesions of other species of *Entyloma* were first examined for 'sporidia', then lesions of fungal parasites other than *Entyloma* were searched for similar 'sporidia'. Finally, the surfaces of leaves not infected by parasitic fungi were examined for such spores.

(1) *Isolations from lesions of* Entyloma. Allantoid 'sporidia' were repeatedly isolated from the surface of lesions of *E. ficariae* (Berk.) Fischer v. Waldh. on *Ranunculus ficariae* L. from one locality in France and two in England; *E. achilleae* Magn. on *Achillea millefolium* L. from one locality in France; *E. bellidis* Kreiger on *Bellis perennis* L. from two localities in France; *E. tanaceti* Syd. on *Tanacetum vulgare* L. from one locality in France; *E. fergussoni* (Berk. & Br.) Plowr. on *Myosotis arvensis* (L.) Hill from three localities in France; *E. serotinum* Magn. on *Symphytum officinale* L. from one locality in France; and *E. eryngii* (Corda) de Bary on *Eryngium campestre* L.

from one locality in France. Both (*a*) and (*b*) allantoid 'sporidia' were isolated from the surface of lesions of *E. ficariae*. In other species the small amount of material examined prevented the identification of different kinds of allantoid 'sporidium'. The 'sporidia' isolated from smut lesions on Boraginaceous hosts, however, included some which were more cylindrical than the commoner curved kind. In addition, 'half-moon-shaped sporidia' were consistently isolated from the surfaces of lesions of *E. fergussoni*, *E. serotinum* and *E. tanaceti*. No needle-shaped 'sporidia' were isolated from the lesions of any of these species.

The chlamydospores formed in the host by these smuts were germinating in the leaf in only three of the species examined: *E. calendulae*, *E. dahliae* and *E. eryngii*; in the others, therefore, chlamydospore germination does not account for the formation of the surface 'sporidia'.

(2) *Isolations from lesions of other fungi.* 'Half-moon-shaped' spores of similar appearance and behaviour to the 'sporidia' isolated from the surface of *Entyloma* lesions were present in the spore prints obtained by suspending leaves infected by other species of smut or with rust fungi over agar. They were present amongst the teleutospores of *Phragmidium fragariastri* (DC.) Schroet. on *Potentilla sterilis* (L.) Garcke, *Puccinia malvacearum* Mont. on *Malva sylvestris* L., and *P. violae* DC. on a garden pansy. A panicle of *Arrhenatherum elatius* (L.) J. & C. Presl., heavily infected by *Ustilago avenae* (Pers.) Rost., was completely mirrored by a deposit of 'half-moon-shaped' spores when it was suspended over agar. These spores, while resembling very closely the 'sporidia' associated with the lesions of *Entyloma*, were also very like those described for *Itersonilia* spp. by Derx (1948) and Olive (1952). Allantoid spores closely resembling type (*b*) were also found in the spore prints of leaves infected by other smuts and by rusts, both beneath the lesions and elsewhere in the leaf print. In appearance and behaviour they approximated to Derx's (1930) description of *Tilletiopsis*. Needle-shaped and (*a*) 'sporidia' were never found in the spore prints from lesions of these fungi.

(3) *Isolations from 'healthy' leaves.* The leaves of as many different species of plant as possible in a piece of meadow land with some trees in the grounds of the Royal Holloway College were collected at fortnightly intervals over 8 months in a range of weather conditions and were suspended over 2 % malt agar for 24 hr., thus allowing for the possible diurnal discharge of spores. The agar surface was examined microscopically for spores and then kept at room temperature for 4 or 5 days until colonies were distinguishable. Monospore isolations were made and maintained on 2 % malt agar.

The leaves examined were taken from the tree, shrub, and ground layers, and the litter of the broad-leaved trees was also sampled. Growing leaves of the following species were sampled: *Agrimonia eupatoria* L., *Arrhenatherum elatius* (L.) J. & C. Presl., *Castanea sativa* Mill., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Glechoma hederacea* L., *Hedera helix* L., *Larix decidua* Mill., *Potentilla sterilis* (L.) Garcke, *Primula vulgaris* Huds., *Quercus robur* L., *Ranunculus ficaria* L., *Rubus* sp. (blackberry), *Rumex acetosa* L., *Stellaria holostea* L., *Taraxacum officinale* agg., *Vicia cracca* L., and *Viola riviniana* Rchb. and litter of *C. sativa*, *F. sylvatica* and *Q. robur*. Some species

were tested consistently throughout the period, others for only part of the time, because the growing period was limited to certain seasons. The number of dead leaves in the litter declined towards midsummer, as the dead leaves rotted, and from August onwards the dead leaves were those of the current season.

Fungi isolated

(1) Species of *Sporobolomyces* are easily distinguishable by their pink colonies which make them at first sight the most striking part of a leaf print, where they often shadow the leaf veins. They are very frequent and were recorded from 152 of the 211 leaves tested. No attempt was made to identify these species.

(2) Equally frequent, though less conspicuous, were the colourless, allantoid, slightly curved spores, answering to Derx's (1930) description of *Tilletiopsis*, which form white to yellow-brown colonies. These were found in prints from 142 of the 211 leaves. Certain of them resembled very closely the (*b*) 'sporidia' isolated from the surface of *Entyloma* lesions.

(3) Finally, from fourteen of the 'healthy' leaves 'half-moon-shaped' spores were isolated. These germinated to give a mycelium with clamp connexions resembling in every way that originating from the germination of 'half-moon-shaped sporidia' isolated from *Entyloma* lesions and of spores isolated from lesions of other kinds of fungi. These isolates approximate to Derx's (1948) and Olive's (1952) descriptions of *Itersonilia*. The fungi of groups (2) and (3) were isolated and studied in greater detail.

Tilletiopsis. The majority of the fungi resembling *Tilletiopsis* consisted of two distinct strains, one with larger ballistospores, resembling the (*b*) 'sporidia' isolated from the surface of *Entyloma* lesions and forming an ivory-white 'frosted' colony, the other with smaller ballistospores forming a yellow-brown colony which later became chocolate-coloured. Occasionally isolates were secured which varied slightly from either of these strains.

Tilletiopsis species with larger ballistospores (Fig. 3*a–e*). A detailed study was made of a monospore culture isolated from the surface of a leaf of *Primula vulgaris* in August 1955. This fungus was abundant throughout the year on the surfaces of living or dead leaves of a wide variety of Angiosperm species.

Ballistospores 12–20 (av. 16) × 2–3 (av. 2·5) μ, borne singly, germinating at both ends immediately after discharge to give a narrow mycelium, 1·5–2 μ wide, sparsely septate (every 125–200 μ) or breaking up into hyaline, straight blastospores, 10–13 × 2–3 μ; chlamydospores, 12–14 μ diam. terminal or intercalary. Colony on malt agar white when young, becoming ivory to deep cream with age, young ones appear 'frosted', older have a dull surface, but the colony is always soft to the touch. Blastospores are more frequently formed in 1- to 2-day-old colonies than in older ones, their abundance imparting to the colony a slimy appearance; other young colonies grow without blastospore formation, resulting in a more 'open', branching mycelium. The former type of growth is more typical of colonies from ballistospores taken from newly subcultured colonies. Formation of ballistospores commences a short distance behind the actively growing margin of the colony. They are

discharged to considerable distances and give rise to scattered daughter colonies beyond the margin of the main colony. Mature chlamydospores are found in the older, central parts of the colony, but hyphal swellings and early stages in chlamydospore formation can be found quite near the margin.

The fungus agrees in all respects with *Tilletiopsis washingtonensis* Nyland (1950), except for minor differences such as the range of size of ballistospores, that given by Nyland being 8–19 × 2–2·6 (av. 14 × 2·4 μ).

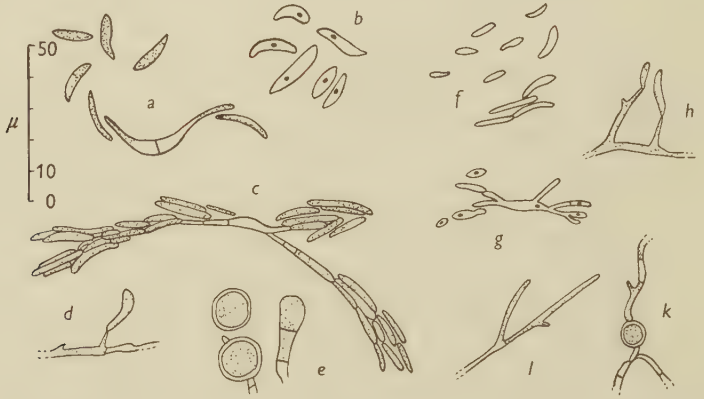

Fig. 3. *Tilletiopsis washingtonensis*. *a*, *b*, Ballistospores and stages in germination; *c*, young colony producing blastospores; *d*, ballistospore formed on a hypha; *e*, chlamydospores. *f–k*, *T. minor*. *f*, *g*, ballistospores and stages in germination; *h*, *i*, mycelium, with ballistospores in *h*; *k*, chlamydospore. [*b*, *g*, Material stained with propiono-carmine.]

Tilletiopsis species with smaller ballistospores (Fig. 3 *f–k*). The description is based on a monospore culture isolated from the surface of a dead oak leaf in April 1955; distribution is the same as that of the preceding species. Ballistospores 5–10 (av. 7) × 1–2 (av. 1·5) μ, borne singly, germinating immediately after discharge at both ends to give further ballistospores or hyaline, straight blastospores, 8–18 × 1–2 μ, or a narrow mycelium, 1–1·5 μ wide, with occasional septa; chlamydospores 8–12 μ diam.

Colony on malt agar light buff when young, becoming yellow-brown to chocolate with age, the surface hardening in cartilaginous flakes. Ballistospores are very frequent in young colonies, giving them a 'broken up' appearance, and assuring a quicker spread over the agar surface than occurs with *T. washingtonensis*, which forms only mycelium or buds in the early stages of growth on malt agar. The first hyphae of the young colonies tend to grow in a curve, and as the tips of the hyphae advance across the medium, the curve may be reversed, giving an undulating appearance to the mycelium. If a coverslip is placed over the developing colony, many side branches are formed; if no coverslip is present, short, pointed sterigmata are formed instead of side branches. As the colony develops, the surrounding medium gradually becomes brown.

With its abundant budding and rarely septate mycelium, this fungus resembles a 'true yeast'. Except for a slight variation in ballistospore size,

this strain of *Tilletiopsis* approximates in most respects to Nyland's description of *T. minor*. The measurements given by Nyland (1950) for the ballistospores of this species are 5·8–14 × 1·5–2 (av. 9 × 1·8) μ.

Itersonilia (Figs. 4, 5). The description is based on a monospore culture isolated from a leaf of *Taraxacum officinale* in November 1954. The fungus was found occasionally throughout the year on the surfaces of living and dead leaves of many Angiosperms, particularly those with hairy leaves; less frequent on 'healthy' leaves in summer but very frequent on the surface of lesions of fungal parasites.

Ballistospores 12–21 (av. 16) × 7–15 (av. 11) μ, 'half-moon-shaped' ($\frac{1}{6}$ segment of a sphere), binucleate, germinating immediately after discharge by one or two germ-tubes, hyphae 3–5 μ wide. Mycelium branching with typical clamp connexions. Intercalary hyphal swellings common, terminal ones occasional, the latter sometimes growing out at the distal end to form mycelium. Chlamydospores 10–15 μ diam., with a thick but hyaline wall and central oil globule, the contents often degenerating with time, plentiful in older mycelium; each has a clamp connexion at its base; germination not observed.

Colony on malt agar white to pale cream, the surface 'frosted' at first, becoming shiny with age. Strong, unpleasant smell on most media.

The mode of germination of the ballistospores varies with the surface on which it occurs. One or more germ-tubes arise from the curved surface, rarely from the straight side. On an agar surface the hypha advances rapidly, the spore soon loses its contents, and simple cross-walls cut off the empty regions at the base. The first clamp connexions are formed early, and thereafter cross-walls are formed only in association with clamps, except in very old mycelium, when simple cross-walls are found. Occasionally, after growing for a short distance, the tip of the germ-tube swells up to form another ballistospore. This is germination by repetition rather than by budding, as a short germ-tube is always formed. On a hard surface, such as a glass slide or the leaf surface, the short germ-tube soon swells at the tip to form a lobed appressorium-like structure with dense contents which stain deeply with cotton blue. A hypha with typical clamp connexions often arises from the surface of this lobed structure and forms a mycelium, if sufficient nutriment is present (Fig. 4*c*, *e*).

The surface of a young colony on 2% malt agar, viewed under the low power of the microscope, appears irregular with an aerial system of 'wisps', formed by the aggregation of hyphae. These aggregations all tend to point in one direction at the same angle to the surface. Ballistospores are frequently formed on the hyphae which make up these aggregations.

If mycelium from an actively growing culture is transferred to liquid malt extract, spherical colonies of clamp mycelium develop, and occasional cylindrical to allantoid cells are formed as outgrowths or buds from the hyphae. These buds vary much in size, measuring 4–10 × 2–5 μ, and are uninucleate (Fig. 5*g*). The further development of these cells was not followed, but they appear to be similar to the 'conidia' described for *I. perplexans* by Olive (1952).

Apart from slight differences in morphology from *I. perplexans* as described by Derx (1948) and Olive (1952), the agreement seems to be

such as to justify its identification as *I. perplexans*. The dimensions of the ballistospores given by Derx are 14–15 × 8–10 μ and by Olive are 12·2–18 × 7·6–10·6 μ. As the walls of the ballistospores are thin, it is

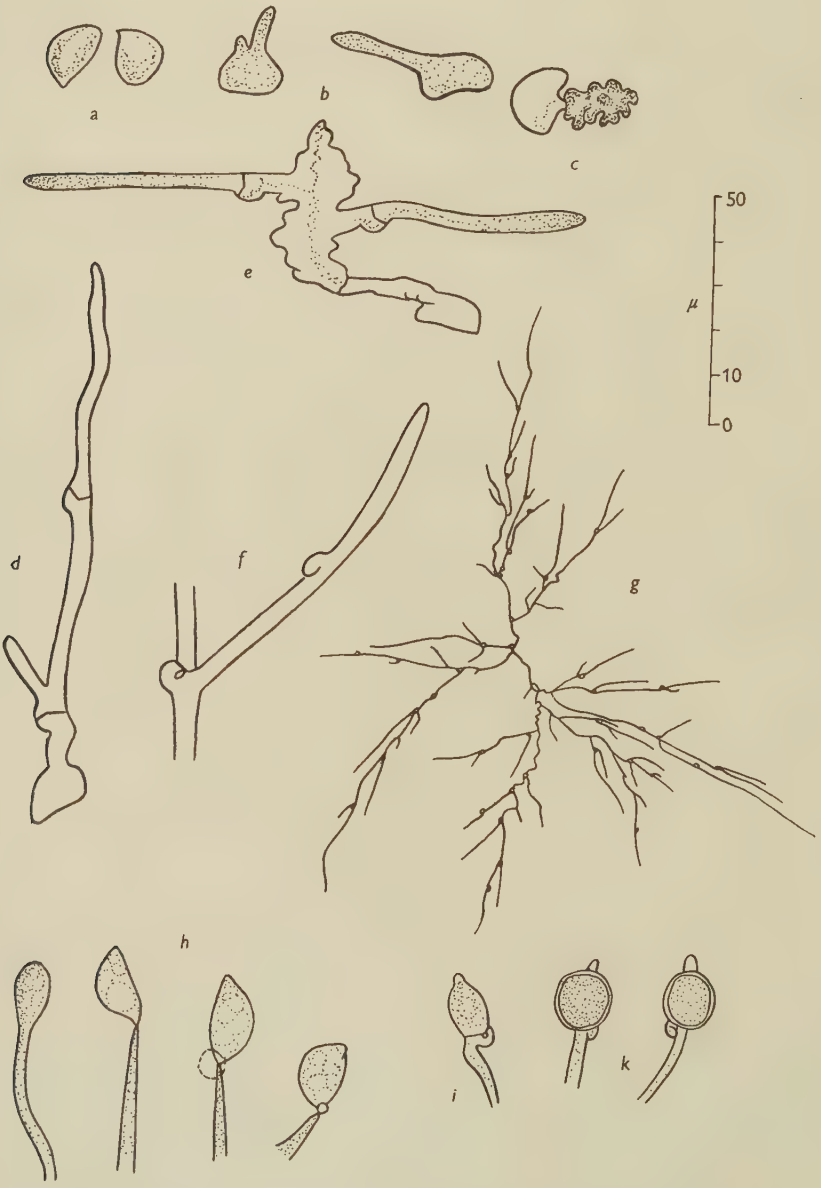

Fig. 4. *Itersonilia perplexans*. *a*, *b*, *d*, Stages in ballistospore germination on agar; *c*, *e*, stages in germination on a hard surface; *g*, young colony from a germinated ballistospore, showing the position of clamp connexions; *f*, clamp mycelium; *h*, ballistospore formation; *i*, *k*, chlamydospore formation, showing clamp connexions at the base of each spore.

possible that the differences in reported dimensions might be accounted for by differences such as the medium employed, the age of the ballistospores, and the fact that different populations were measured by each author. Olive describes the formation of a swelling, the 'sporogenous cell' from which grows out the 'sporophore' which eventually produces the ballistospore at its tip. Although such structures were occasionally found in the material examined, they were by no means so constant a feature as Olive's description suggests. The ballistospores are usually produced at the ends of tapering hyphae 30–100 μ long which start as simple aerial branches of the mycelium. The ballistospores are too large for *I. pyriformis* Nyland (1949), and the almost invariable formation of a clamp connexion with each chlamydospore agrees with *I. perplexans* rather than *I. pyriformis*. Since Olive suggests that *I. pyriformis* is probably a variant of *I. perplexans*, it was decided to assign the fungus isolated to *I. perplexans*.

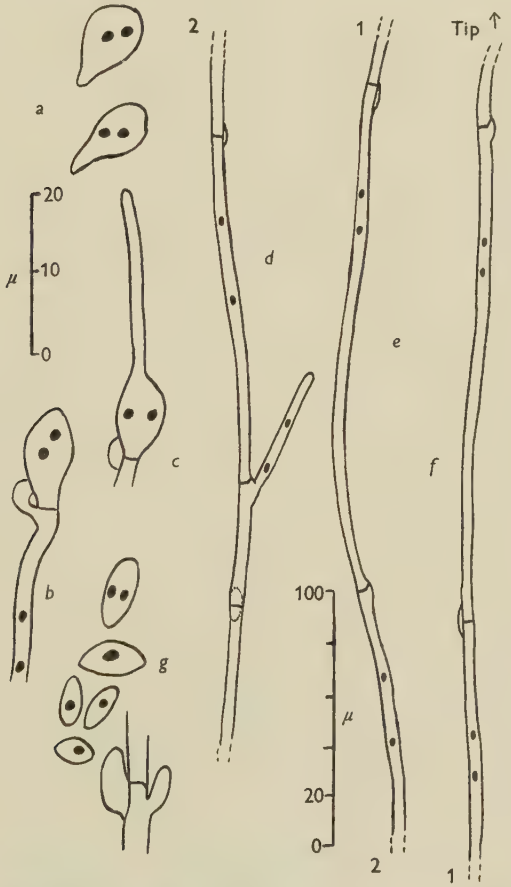

Fig. 5. *Itersonilia perplexans*. Drawings from preparations stained with propiono-carmine. *a*, Freshly fallen ballistospores, showing nuclei; *b*, *c*, 'sporogenous' cells, or young chlamydospores, with clamp connexions at their bases; *d–f*, single hypha showing clamp connexions and paired nuclei; *g*, 'conidium' production, showing nuclei.

Discussion

The life cycles of the Entyloma *species*

It is difficult to obtain conclusive proof that *I. perplexans* and *T. washingtonensis* are not part of the life cycle of *Entyloma*. The evidence presented here is mainly negative, and the widespread occurrence of both fungi in situations other than on *Entyloma* lesions might be a manifestation of the ability of particular states of *Entyloma* to lead an independent existence. Neither fungus was produced when the chlamydospores of *Entyloma* germinated, nor formed by the vegetative parasitic mycelium. Inoculation experiments were negative, but so were all inoculations using allantoid (*a*) 'sporidia', as well as a large proportion of those using needle-shaped 'sporidia'. A positive piece of evidence where *I. perplexans* is concerned is the great difference in appearance between the clamp mycelium formed by *I. perplexans* in culture and the parasitic mycelium of the *Entyloma*. The mycelium formed in culture by germinating chlamydospores of *Entyloma* does not form clamps, and is very unlike the saprophytic clamp mycelium of *I. perplexans*. Taken in its entirety, the evidence seems sufficient to exclude *I. perplexans* and *T. washingtonensis* from the species of *Entyloma* examined.

There are still very many gaps in the accounts of the life cycles of *E. calendulae* and *E. dahliae*, especially concerning the association of nuclei in the mycelium. The, presumably haploid, nuclei from the promycelial branches associate in pairs in the needle-shaped sporidia. These needle-shaped sporidia are capable of infecting the host. They germinate in culture to give a twisting mycelium which forms more needle-shaped sporidia and allantoid sporidia, the number of nuclei in which was not ascertained. Allantoid 'sporidia' isolated from the surface of lesions, however, were always uninucleate, and it appears that a dissociation of nuclei, such as is described by Churchward (1940) for *Tilletia caries*, must occur. If so, it is difficult to interpret the fact that fusions between allantoid 'sporidia' were not obtained, unless the requirements for this fusion were not fulfilled in the conditions of the experiments. Rediploidization must occur at some point before chlamydospore formation, and it is most likely that it takes place at some time before or early in the parasitic phase, as clamp connexions occur in young lesions. The nuclei in these clamps were not observed, but the connexions themselves appeared normal and were not 'pseudoschnallen' (Harder, 1927). Clamp connexions were not seen in *Entyloma* mycelium in culture, whether in mycelium from germinated chlamydospores, needle-shaped sporidia or allantoid (*a*) sporidia. Thus, if the association of nuclei begun in the needle-shaped sporidia is permanent, there must be two kinds of dikaryotic mycelium, one saprophytic and without clamps, and one parasitic with clamps.

Neither of these species of *Entyloma* has been induced to complete its life cycle in culture by forming chlamydospores. Infection of *E. dahliae* has, however, for the first time, been brought about in the host by inoculation with a single needle-shaped sporidium, resulting in the formation of a lesion with viable chlamydospores.

The bearing of this investigation on past records

The similarity between the ballistospores of *Tilletiopsis washingtonensis* and the allantoid sporidia of *Entyloma* has caused confusion in the descriptions of sporidial stages of *Entyloma*. The varied descriptions of sporidia given in the literature, even for one species of *Entyloma*, have, in some instances, been due to the presence of *Tilletiopsis* on the lesions. Thus Hanna's (1938) description of large and small 'sickle-shaped conidia' in *E. nymphaeae* can probably be explained by the presence of one or more strains of *Tilletiopsis* on the surface of the lesions. Hanna does not give measurements of his 'conidia', but the dimensions of his figures of the smaller strain agree with those of *T. minor*; he also found that this strain made potato-dextrose agar turn dark brown. *T. minor* forms a brown colony and a soluble brown pigment.

The allantoid sporidia of *E. ficariae* figured by Ainsworth & Sampson (1950, fig. 1*a*, p. 22) suggest the budding stage of germinated ballistospores of *T. washingtonensis* and probably represent this fungus. Stempell (1935) describes how 'mycelium I', formed by his 'crescent-shaped conidia' of *E. ficariae* and *E. calendulae*, formed abundant chlamydospores in culture, and it is possible that he was dealing with a species of *Tilletiopsis*.

Although both Derx (1948) and Olive (1952) consider that the 'mycelium II' described by Stempell as part of the life cycle of *E. calendulae* really represented an independent fungus, *I. perplexans*, neither author compared his isolate with material from a lesion of *E. calendulae*, but relied on Stempell's published description. This comparison has been made in the present investigation, where isolates from the surface of lesions of *E. calendulae* and from other sources have been found to correspond. *I. perplexans* is, however, so frequent on the surface of lesions of species of *Entyloma*—especially those which appear during the latter part of the year—that it is strange that it has not been described more often from these sources. The only published records of a fungus approximating to *I. perplexans* in association with lesions of *Entyloma* are those of Stempell (1935) for *E. calendulae*, Ainsworth & Sampson (1950) for *E. calendulae* and *E. dahliae*, where fig. 1, *e–j*, p. 22 undoubtedly represents *Itersonilia*, and the account of Brady (1953), in which 'half-moon-shaped sporidia' were reported from *E. calendulae*, *E. dahliae*, *E. tanaceti*, *E. serotinum* and *E. fergussoni*, and were illustrated for *E. calendulae*. This paper was a report of the preliminary stages of the present investigation, and the 'sporidia' described there were those isolates of *I. perplexans* which are studied further here. Recently, Wilkinson (1952) and Sowell (1953) in America, and Channon (1956) in this country, have found evidence indicating that *Itersonilia perplexans* is a causal agent of parsnip canker.

This work was carried out with the help of a Tutorial Research Studentship at Royal Holloway College during 1949–51, and of an Exchange Scholarship from the Societé Nationale de la Recherche Scientifique held at the Institut National Agronomique, Paris, in 1951–52. Thanks are extended to the authorities of these institutions for their generosity in providing facilities for research, to Dr M. A. P. Madge of Royal Holloway

College for her valued help and criticism, and to Prof. G. Viennot-Bourgin of the Institut National Agronomique and Miss K. Sampson for their encouragement throughout the course of study. The work forms part of a thesis approved for the degree of Ph.D. in the University of London.

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(*Accepted for publication* 26 *March* 1959)

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SOME EFFECTS OF WATER AND ANAEROBIC CONDITIONS ON *FUSARIUM OXYSPORUM* F. *CUBENSE* IN SOIL

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The behaviour of *Fusarium oxysporum* f. *cubense* in non-sterile soil under various environmental conditions was studied by a baiting technique. The bait used was pieces of sterilized banana leaf placed in the soil. Carbon dioxide inhibited the production of chlamydospores, as did flooding the soil containing the fungus. The general attenuation of activity that took place over a period of 3 months in these conditions is attributed to the lack of resistant chlamydospores, this reducing the ability of the fungus to survive in the inactive condition. Short periods of exposure to these conditions stimulated the germination of spores in the soil.

Introduction

During the past few years, knowledge of the saprophytic behaviour of fungi in soil has greatly increased. In spite of this there are many phases still to be studied.

The fact that *F. oxysporum* f. *cubense* (E. F. Smith) Snyder & Hansen causes a severe disease of the banana plant (*Musa sapientum*) has been known since 1904 (Higgins, 1904), but only in recent years has a study of its saprophytic behaviour in soil been undertaken. Soil moisture content has received much attention, mainly because this is relevant to the practice of flood-fallowing used as a control measure on soils infested by *F. oxysporum* f. *cubense*.

Previous workers have studied the survival of *F. oxysporum* f. *cubense* in soil under varying conditions, determining survival by removing some of the infested soil from the given environment and placing it under conditions favourable to growth. This constitutes a basis for criticism of much previous work, since it tells us nothing about the potential activity of the fungus under the chosen conditions. In the present work it is the ability to become active rather than the mere survival in the experimental treatments which has been measured.

Materials and methods

The soil used in all these experiments was a light, sandy loam, pH 4·15, from Styal Woods, Cheshire. This was collected, taken to the laboratory in polythene bags, and there sieved and kept moist in tall glass jars. This soil has not been found to possess any indigenous species of *Fusarium*. In these experiments the soil was inoculated by adding 1 ml. spore suspension of *F. oxysporum* f. *cubense* to each 50 g. soil; in this article, 'inoculated soil' refers to soil infested in this way.

The fungus used throughout this work was an isolate of *F. oxysporum* f. *cubense* (E. F. Smith) Snyder & Hansen obtained in 1957 from diseased banana plants in the Cameroons, West Africa, by Dr A. G. Prendergast. This was transferred to several malt slopes when received in the laboratory, and slope cultures used for inoculum were in no case more than twice removed from the original. This reduced the possibility of a change from the original pathogenic isolate to a more saprophytic one.

It has been stated that the intention was to measure the potential activity of the fungus in the soil, and not just its survival. A baiting technique, in which small pieces of organic material are left in the soil for a period, and then surface-sterilized and plated on a medium suitable for growth, was chosen for this purpose. If *F. oxysporum* f. *cubense* grows out of the baits when they are placed on agar, then not only has it survived in the soil, but it has been able to grow into the pieces and colonize them under the conditions obtaining in the experimental soil.

Four types of organic material were tested for their suitability as baiting material. These were: (*a*) oak leaf, (*b*) cocksfoot leaf (*Dactylis glomerata*), (*c*) potato tuber, (*d*) banana leaf. Of these four, banana leaf was colonized most readily by the fungus and, as it was easy to handle, it was used as the baiting material throughout.

For each of three replicate treatments, twenty-five small pieces of banana leaf (5 mm. square) were sterilized for 24 hr. in propylene oxide vapour. These pieces were then placed on the soil to be tested and left for 4 days. At the end of this time they were lifted from the soil, wrapped in a square of muslin, and surface-sterilized in 7 % calcium hypochlorite solution (2·5 % available chlorine) for 15 min. After washing in six changes of sterile water, the pieces were allowed to dry thoroughly and then plated on 3 % malt-extract agar. After 4–5 days *F. oxysporum* f. *cubense*, if present, grew out and gave a characteristic deep pink colour, so that counting the number of pieces colonized by the fungus gave little difficulty. The colonization figure given in the tables is a corrected mean from all replicates.

In most cases the baits could readily be placed on or removed from the surface of the moist soil in Petri dishes or wide-necked flasks. However, in those experiments where the soil was placed under water in wide-necked 100 ml. flasks, difficulty was found in placing the banana pieces on the soil. This was overcome by laying the baits flat on a single layer of clean washed muslin, the ends of which were folded over to make a flat envelope, which was then placed on the surface of the submerged soil, the corners of the envelope being pressed into the soil to hold it in place. Early tests showed that the fungus was well able to colonize the pieces through the muslin and, provided the baits were well spaced, there was no evidence of colonization from adjacent baits during the 4 days.

Some of the experiments involved the maintenance of different gaseous mixtures over the soils. The containers of soil were placed in desiccators with well-sealed lids and provided with closable entrance and exit tubes for the gases. In some experiments an atmosphere of air with the oxygen removed from it was obtained by using pyrogallol solution in caustic soda in the lower half of the desiccator.

For morphological studies of the fungus in soil under the experimental

conditions, the method of Legge (1952) was used. Sterilized glass tapes were placed on agar plates of the fungus and incubated at 25° C. for 5 days, when a strong growth of hyphae plus conidia had penetrated each tape. The tapes were buried in *c.* 1 cm. of fresh moist soil in Petri dishes placed under the conditions to be tested. In the case of soil under water, the tapes were buried in soil in 100 ml. flasks to which 5 cm. depth of water was added. Tapes were lifted at intervals, stained for 2 min. in hot phenolic rose-bengal, and mounted in dilute glycerine. Since only the viable, or recently viable, parts of the fungus became stained, it was possible to determine microscopically the condition of the fungus in the soil.

Experimental work

Preliminary experiments showed that the fungus could survive in the alien soil for periods of at least 8 months. It was also found that the fungus could, from the soil, colonize a range of sterilized and fresh materials. This suggests that *F. oxysporum* f. *cubense* is a good competitive saprophyte.

The main aspect under consideration here is the effect of some environmental conditions on the saprophytic ability of the fungus in soil.

Effect of submergence on activity of Fusarium oxysporum *f.* cubense

This experiment was designed to determine whether *F. oxysporum* f. *cubense* could survive in the flooded soil and for how long it could tolerate these conditions. Soil (50 g.) was placed in each of twelve 100 ml. flasks, which were then inoculated with the fungus. Series A was left moist;

Table 1. *Effect of submerging soil on activity of* Fusarium oxysporum *f.* cubense

	Percent colonization after				
	1 week	2 weeks	4 weeks	8 weeks	12 weeks
A. Moist soil	66	52	45	51	53
B. Soil + tap water	50	46	18	10	4
C. Soil + sterile water and paraffin	60	49	40	6	3
D. Soil + aerated water	56	33	17	5	2
Mean colonization in all treatments under water	55	42	25	7	3

series B had tap water added to a depth of 5 cm. above the soil surface (water level kept constant throughout the experiment); series C had recently distilled sterile water added and then 1 ml. liquid paraffin poured on top in order to inhibit the solution of oxygen in the water; the flasks of series D were flooded to a depth of 5 cm. with water that was kept aerated by means of an air pump. All flasks were plugged with cotton-wool and incubated on the laboratory bench (17–20° C.). The flasks were tested by baiting after 1, 2 and 4 weeks, and then once each month (Table 1).

The percentage colonization of the baits by *F. oxysporum* f. *cubense* in the flooded soils (Table 1) became gradually lower, until after 12 weeks very few pieces were colonized. Two possibilities might account for this attenua-

tion of the fungus. Either there is a gradual accumulation of some toxic substance to a threshold level at which the fungus is affected, or the generally unfavourable conditions continually deplete the viable spore population of the fungus through the term of the experiment. The flasks were re-inoculated (see the fourth experiment) after 3 months in order to resolve this problem.

The decline under flooded soils was much greater than that in the moist 'control' soil. However, there was no important difference between the aerated and non-aerated flasks, which indicates that the decline in the potential activity of *F. oxysporum* f. *cubense* was not specifically due to any limitation of oxygen availability.

Effect of different gases on activity of Fusarium oxysporum *f.* cubense

Triplicate malt agar slopes inoculated with the fungus and lightly plugged with cotton-wool were placed in each of four desiccators. One was left open to the air; another had the oxygen removed from the air in it; another was filled with nitrogen; the fourth was filled with carbon dioxide. The atmospheres were renewed every third or fourth day when the slopes were examined for growth of *F. oxysporum* f. *cubense*.

After 1 week there was strong growth of the fungus in all four series, but only in nitrogen and in normal air was the mycelium coloured pink. After 2 weeks the amount of mycelium had increased in all four series and in each the fungus had the pink pigmentation. Microscopic examination showed that morphology was normal in each treatment.

Table 2. *Activity of* Fusarium oxysporum *f.* cubense *in soil under nitrogen, carbon dioxide, and air minus oxygen*

	Percentage colonization after		
	1 week	4 weeks	6 weeks
Air	75	52	51
Nitrogen	73	66	56
Air minus oxygen	72	36	35
Carbon dioxide	69	9	10

Although composition of the atmosphere did not affect the growth of the fungus on agar, it seemed possible that it might yet have an effect on the fungus in soil. In order to investigate this, twelve Petri dishes were filled to a depth of *c.* 1 cm. with fresh moist soil, which was then inoculated with the fungus. The dishes, with the lids slightly raised to ensure circulation of the gases, were placed in the four atmospheres described above, and the soil tested by baiting after 1, 4 and 6 weeks. Each time the desiccators were opened, the atmospheres were recharged appropriately for the series. Percentage colonization of the baits is given in Table 2.

The percentage colonization figures given in Table 2 show that the fungus was potentially active in all the plates even after 6 weeks, although some reduction in percentage colonization was noticeable. This was most marked in the soil under carbon dioxide. These experiments indicate that

carbon dioxide plays a greater part in attenuation of the fungus than does lack of oxygen.

For a long-term experiment, small tubes (1 in. diam. × 2 in. long) were filled with moist, inoculated soil. These were placed for 4 days in an atmosphere lacking oxygen, then quickly corked and completely sealed with a layer of wax. Two tubes were opened and tested after 1, 2, 4, 8 and 12 weeks, respectively, for the presence of the fungus by direct droplet-plating of crumbs of soil (Park, 1958). The fungus appeared on the plates after 8 weeks in soil but not after 12 weeks. The remaining tubes were then opened to the air for several days, after which it was easy to demonstrate that *F. oxysporum* f. *cubense* was present in the soil. *F. oxysporum* f. *cubense*, then, was not eliminated in this time by anaerobic conditions but after 12 weeks its population had declined to a level not detectable by droplet plating.

Effect of different gases on submerged Fusarium oxysporum *f.* cubense

The aim of this experiment was to determine whether the different gases had a more marked effect when the fungus was submerged under water. Six flasks, each containing 50 g. inoculated soil, were flooded to a depth of 5 cm. with boiled tap water, lightly plugged with cotton-wool and placed in desiccators, which were then filled with nitrogen or with carbon dioxide. The atmospheres in the desiccators were renewed twice each week. As nitrogen and air without oxygen both had the same effect on the fungus in soil, the atmosphere of nitrogen was chosen as an oxygen-deficient control for comparison with carbon dioxide. Percentage colonization of the baits by the fungus is given in Table 3.

Table 3. *Effect of nitrogen and carbon dioxide on activity of* Fusarium oxysporum *f.* cubense

	Percent colonization after						
	1 week	2 weeks	4 weeks	7 weeks	9 weeks	12 weeks	16 weeks
Soil + water and nitrogen	75	80	55	14	12	*	*
Soil + water and carbon dioxide	60	20	9	10	*	14	1

* No determination made.

The activity under carbon dioxide was high at first, but after 2 weeks there was a sharp drop to a low level of colonization and after 4 months only one piece out of seventy-five was colonized. Nevertheless, the ability of *F. oxysporum* f. *cubense* to be active under such conditions is quite surprising. As the fungus was not killed outright, it was concluded that carbon dioxide was not immediately toxic to it. A notable feature of these results is the sudden reduction in potential activity caused by this gas in comparison with the absence of any immediate effect under nitrogen, in which there was a more gradual attenuation of activity.

Since in flooded soil there must be a considerable production of carbon dioxide by anaerobic organisms, it seems likely that this factor plays an important part in the attenuation of *F. oxysporum* f. *cubense* in submerged soil both in the laboratory and in the field.

Re-inoculation of flooded soil with different concentrations of inoculum

The nine flasks from the first experiment described above, the soil of which had been under water for 3 months before *F. oxysporum* f. *cubense* almost disappeared, were used in this experiment. Three levels of corn-meal-sand inoculum (5, 10 and 20 %) were added to the soil in the flasks, in order to find out whether a low level of inoculum would last as long under water as a higher level of inoculum. The inoculum was thoroughly mixed with the soil and the flasks re-flooded with the same water to a depth of 5 cm. above the soil surface. The soil was tested at intervals for the activity of *F. oxysporum* f. *cubense* (Table 4).

Table 4. *Effect of re-inoculation of flooded soil with* Fusarium oxysporum *f.* cubense

Additional inoculum to flooded soil	Percentage colonization after				
	1 week	2 weeks	4 weeks	8 weeks	11 weeks
5%	84	93	84	40	29
10%	88	93	92	60	30
20%	100	100	97	84	50

At first the activity of the fungus was high in all flasks. Throughout the experiment there was a distinct difference in the activity of the three levels of inoculum and this difference was greatest after 8 weeks. After 11 weeks the gap was narrowing a little and all the flasks showed a relatively low level of activity.

The fact that the fungus inoculum was active over a second 3 months eliminates the hypothesis that a specific toxin had accumulated during the first period of flooding. As the decline in activity was gradual, the proposal that general unfavourable conditions slowly reduce the number of viable spores in the soil seems reasonable, and the data agree with the concept that a greater number of spores in the soil leads to a better survival of *F. oxysporum* f. *cubense*.

Morphological observations on Fusarium oxysporum *f.* cubense *in soil under various conditions*

All the previous experiments have shown that *F. oxysporum* f. *cubense* can survive and be active in alien soil, but when the soil is flooded or placed in certain gaseous mixtures, then there is a reduction in activity and, after 4 months, few spores remain alive. It seemed desirable to use a direct method to determine what was happening to *F. oxysporum* f. *cubense* in soil. Infested glass tapes (see under 'Methods', above), buried in soil in Petri dishes, were placed in atmospheres of air, nitrogen, carbon dioxide, and air from which the oxygen had been removed. Tapes were also buried in soil submerged under 5 cm water, and further tapes were placed in sterile water without soil. Two glass tapes were lifted from each treatment for microscopic examination every third or fourth day (Table 5).

In soil under normal conditions, *F. oxysporum* f. *cubense* lived as hyphae,

microconidia and macroconidia up to 10 days, after which there was a gradual decline in viable hyphae and conidia until, 31 days after placing the tapes in soil, none could be found. After 5 or 6 days, chlamydospores were formed and by the tenth day there were many mature ones. These were found on every tape, in a viable condition, until the end of the experiment. Some conidia germinated in the third week and gave a little new mycelium but this soon produced chlamydospores.

Table 5. *Morphological observations on* Fusarium oxysporum *f.* cubense *in soil under different conditions*

Time in soil (days)	Soil and infested glass tapes in atmospheres of: Air				Air minus oxygen				Carbon dioxide				Nitrogen				Flooded soil				Sterile water (no soil)			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
3	4	0	0	0	4	0	0	0	4	0	0	0	4	0	0	0	4	0	0	0	4	0	0	0
7	4	2	3	0	4	1	0	0	4	1	0	0	4	1	0	1	4	1	0	1	4	1	0	1
10	4	3	4	3	4	1	1	1	4	1	1	1	4	1	0	2	4	2	0	1	4	1	0	1
14	2	4	4	2	4	1	1	4	4	1	1	2	3	1	1	2	4	1	1	1	3	2	3	1
19	1	4	4	2	2	1	2	2	4	1	1	2	3	2	2	3	4	1	1	2	2	3	4	1
25	1	4	4	1	2	3	3	1	3	1	1	2	3	3	3	3	3	1	1	1	2	3	4	1
31	0	3	4	0	1	2	3	2	3	1	1	2	2	3	4	1	3	1	1	1	2	3	4	1
37	0	2	4	0	1	2	3	1	3	1	1	2	1	2	4	0	3	1	1	1	0	2	4	1

A = normal conidia; B = autolysed hyphae and young chlamydospores; C = mature chlamydospores; D = germinating conidia.

For each category printed as A–D above, the frequency on the glass tapes is indicated thus: 1 = scarce, 2 = few, 3 = frequent, 4 = abundant.

Of the tapes in the remaining treatments, those in sterile water without soil gave the result closest to that of tapes in fresh soil. Again there was a gradual reduction in the numbers of conidia and a corresponding increase in the numbers of chlamydospores, which were first found after 14 days.

In soil under nitrogen there was a preponderance of viable conidia up to the twenty-fifth day, and then there was a slight decline. Chlamydospores were late in being formed but after 25 days there was a rapid rise in their numbers, until at 37 days they formed the main fungal structure on the tapes. The anaerobic conditions of air minus oxygen gave similar results to the anaerobic conditions of nitrogen. Again chlamydospores were late in forming but, once formation had started, large numbers were found.

The remaining two conditions, namely, soil under carbon dioxide and soil under water, gave results similar to each other, and contrasting markedly with the foregoing observations. In both there were numerous viable conidia remaining even after 37 days, although the number was declining slowly, in part due to the germination of conidia throughout the period of observations. The most notable feature was that very few chlamydospores formed, the few that were formed being small and taking the stain only faintly.

Discussion

From the morphological study of *F. oxysporum* f. *cubense* in soil under these conditions, it is concluded that the normal survival of the fungus in soil after 2 weeks or more depends on the production of chlamydospores. These can remain viable in the soil for long periods and it is these which germinate and colonize any organic material in the soil. Although in an atmosphere of nitrogen, and in air minus oxygen, the formation of chlamydospores is delayed, they are finally produced in large numbers and so, here also, the survival and activity of the fungus is dependent on them. Behaviour in soil under water or in carbon dioxide is very different. Here only an occasional chlamydospore is formed, but at first there are many conidia and the potential activity of the fungus in soil depends on these. In the majority of cases, however, conidia are not known to be very long-lived in soil; consequently, while a fungus dependent on conidia for survival will be able to survive short unfavourable spells, it will not be equipped to survive long periods under such conditions. In some experiments not detailed here (Newcombe, 1958), it has been found that the fungus will live in soil under water or carbon dioxide for only 3 months, but in fresh soil it will live for at least 8 months. Chlamydospores are therefore probably of more significance than are conidia in respect of survival of a fungus in the soil. Carbon dioxide inhibits chlamydospore formation and therefore, even though the fungus can tolerate short periods of high carbon dioxide concentration, its long-term survival in such conditions is affected.

Carbon dioxide keeps *F. oxysporum* f. *cubense* for short periods in the form of conidia which are continually germinating and therefore in a condition to colonize any available substrate. This effect of stimulating the activity and spore germination of *F. oxysporum* f. *cubense* can be either advantageous or not to the long-term survival of the fungus in the soil. If there is much organic material ready to be colonized in the soil—as there is in a banana plantation—then increased germination of spores will lead to multiplication and spread of the fungus. On the other hand, in a soil containing little organic material, e.g. a fallow, flooded field, there is a shortage of suitable substrates for the spores to colonize after they have germinated, and in this case increased germination due to a high carbon dioxide content is unfavourable for survival, in that it leads to a reduction in the number of viable spores remaining in the soil. Dobbs & Hinson (1953) came to the conclusion that there was a fungistatic factor present in soil which prevented spore germination. Carbon dioxide seems to overcome the fungistatic action of the soil on *F. oxysporum* f. *cubense*, so allowing the spores to germinate.

As both the morphological observations and the experimental results of the effects of flooding and high carbon dioxide concentrations on *F. oxysporum* f. *cubense* are similar, it is concluded that the main factor in the elimination of the fungus by flood-fallowing is a high carbon dioxide content in the submerged soil, together with a decreased availability of colonizable substrates. The hypothesis is that this gas keeps the fungal spores germinating without there being any formation of resistant chlamydospores or any replenishment of inoculum so that in a few weeks no viable

spores are left. Under plantation conditions, carbon dioxide would not necessarily reduce survival, as fresh substrates in the form of host plant and plant parts are continually becoming available, but in flooded soil no fresh inoculum is added and so the fungus does not survive well. The length of the flooding period must be long enough to allow a high carbon dioxide level to develop and for this to affect the fungus. The effect on *F. oxysporum* f. *cubense* is not immediate, but is effective over a long enough period.

While the experiments described here indicate that carbon dioxide is an important factor in the elimination of the fungus from flood-fallowed soil, they do not rule out the simultneousa operation of other factors under these conditions.

My thanks are due to Dr D. Park for supervising this work, which was carried out during the tenure of an award from the Department of Scientific and Industrial Research.

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(*Accepted for publication* 7 *April* 1959)

Trans. Brit. mycol. Soc. **43** (1), 60–64 (1960).

APLANOPSIS SPINOSA SP.NOV.

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(With Plate 2 and 2 Text-figures)

Aplanopsis spinosa sp.nov. is described and illustrated, and briefly compared with *A. terrestris* Höhnk.

Aplanopsis spinosa sp.nov.

Mycelium flocculentum extensum. Hyphae basales admodum crassae, ramosae, 40 μ diam., abruptae attenuatae; ramificatis plerumque sympodiacis, ramis novellis sub apicibus hypharum exortis, quippe quae solida quadam ei refrangibili materie impleantur ceterum apices hypharum angusti, acuti. Gemmae et zoosporangia nulla. Oogonia sphaerica vel subsphaerica, spinis dense obtecta, plerumque 25–29 μ diam. (spinis inclusis); oogonia vel in ramis longis, lateralibus, tenuibus edita, vel intercalaria, vel terminalia. Ubi intercalaria vel terminalia tunc oogonia maiora. Antheridia pro quoque oogonio singula, androgyna vel semi-hypogyna. Antheridiorum cellulae clavatae, parieti oogonii lateraliter adpressae. Canales seminarii suppetentes. Oosphaerae maturantur. Oosporae singulae, subcentricae, oogonium implentes, plerumque 14–17 μ diam. Germinatio oosporarum observata. Habitat in pascuorum udorum solis: Pinetum Bedgeburiensis, Kent, England.

The descriptive details were compiled from cultures grown under the following conditions: the substrate, a boiled hemp seed, was inoculated with a single hypha and kept in a Petri dish in glass-distilled water of pH approximately 6·8 and depth approximately 0·5 cm.; some cultures were kept at 10° C., others at approximately 19–20° C.

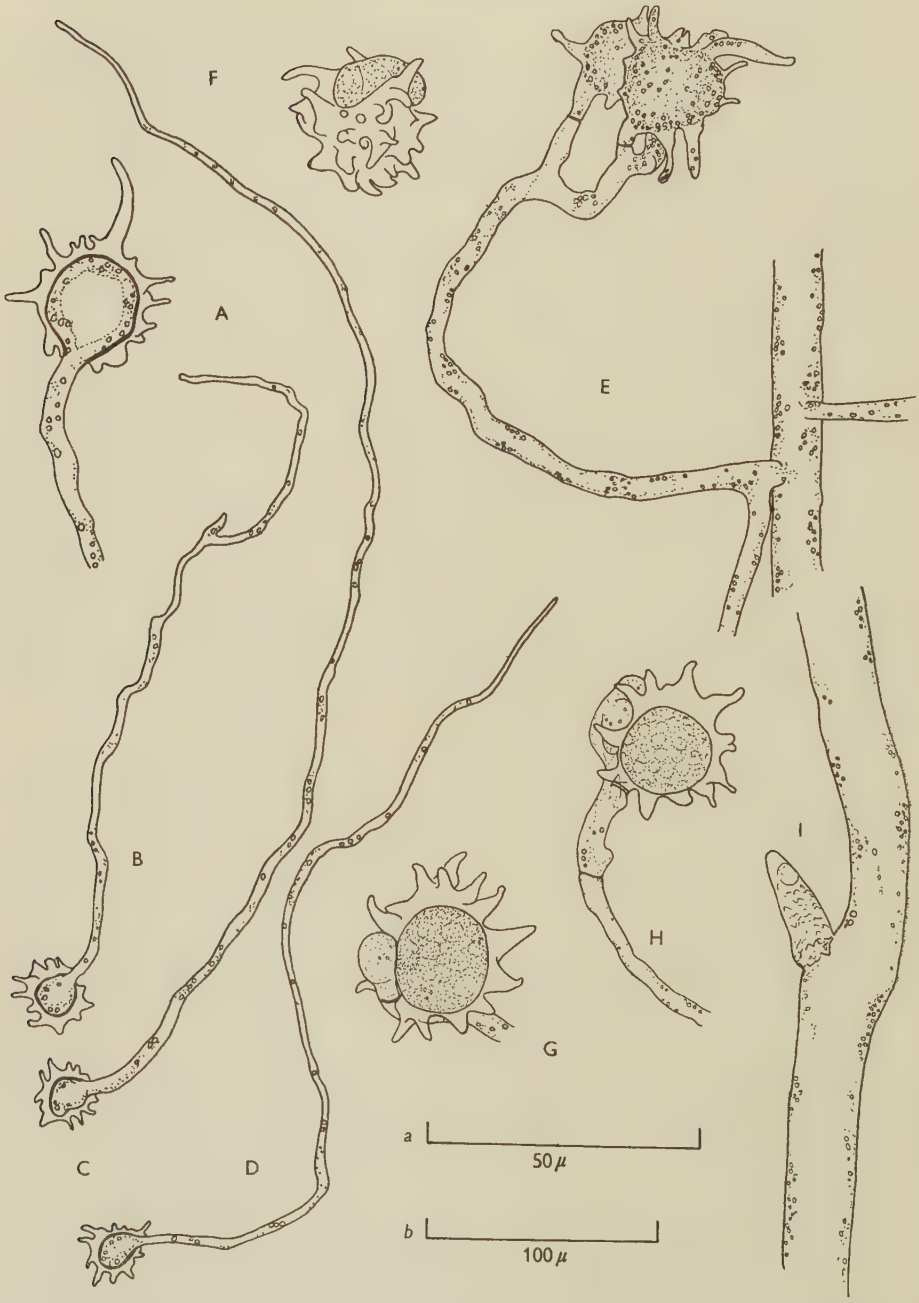
Oogonium and oospore measurements are based on counts from 300 oogonia from three different cultures.

Mycelium flocculent, extensive, reaching 5–6 cm. in diameter on hemp seed in 2 weeks. Basal hyphae relatively thick, commonly 80–100 μ, but up to 150 μ diam.; branching frequently and quickly tapering to about 40 μ diam. Lateral hyphae arising almost at right angles to the main hyphae, sparingly branched. Growth to a greater or lesser extent sympodial, resulting from renewal of growth from below hyphal tips which become filled with a plug of solid refractive material. These are evident in older parts of the turf as short peg-like structures (Text-fig. 1 I); similar plugging may occasionally be found in the main hyphae and near the bases of branches. Hyphal tips otherwise narrow, pointed. Gemmae and zoosporangia absent. *Oogonial branches* produced laterally after 6 days on distal parts of some principal hyphae (oogonial hyphae) where these are approximately 10–20 μ diam. Oogonial branches slender, approximately 5 μ diam., variable in length, the majority between 180 and 320 μ; developed in great abundance giving a furry appearance to the main oogonial hyphae (Pl. 2, fig. 1) and often developed in groups about 150 μ

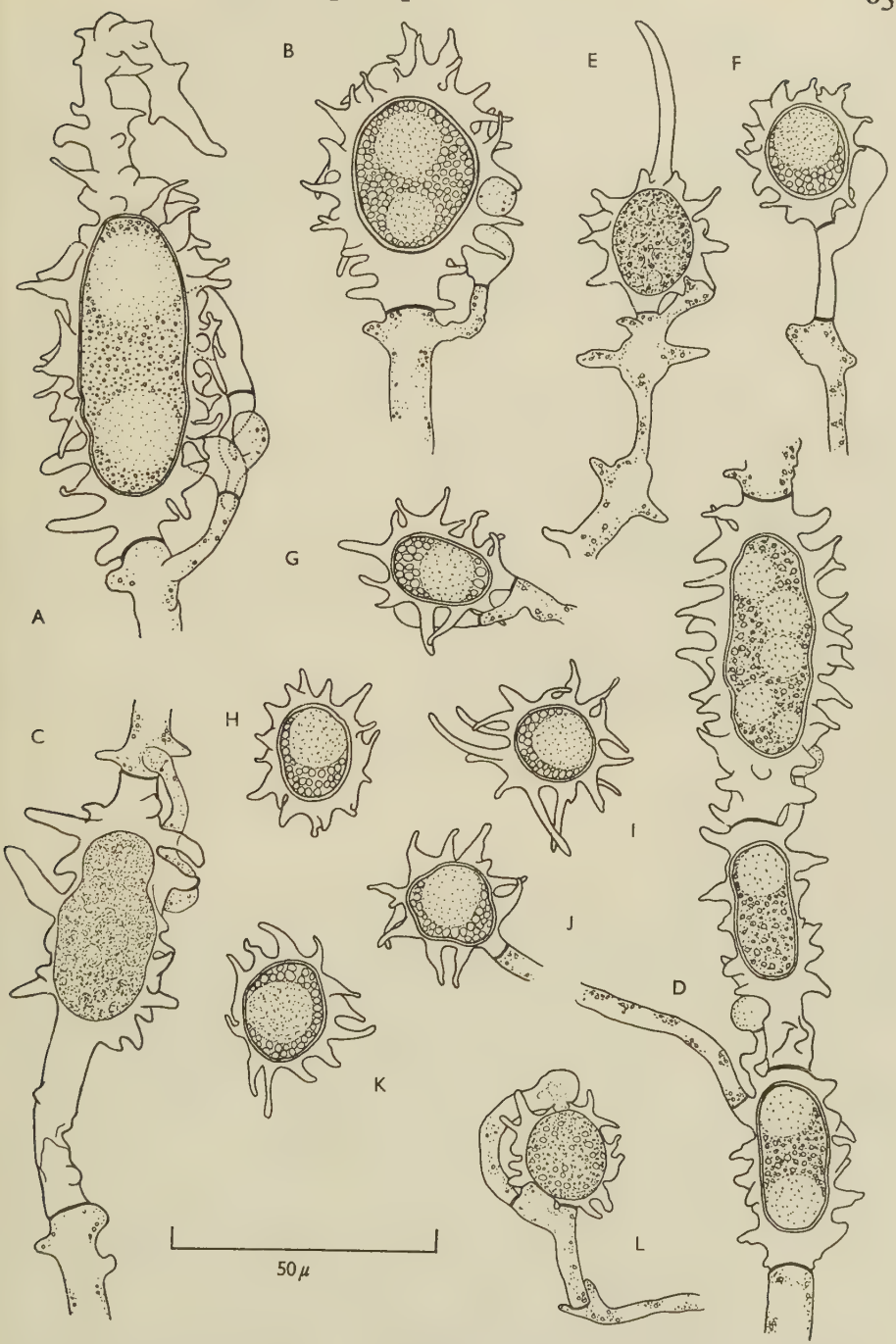
apart, giving a whorled appearance to the oogonial hypha (Pl. 2, fig. 2). *Oogonia* abundant, formed terminally on many of the oogonial branches, intercalary and terminal on the main oogonial hyphae. Lateral oogonia spherical or subspherical, densely covered with mammiform, papillate or tuberculate projections, when mammiform often curved or hooked (Text-fig. 2H, I, J); sometimes with an elongate tubular apiculus (Text-fig. 2E) which rarely may be converted to a hyphal segment by a cross-septum. This feature may also be found on papillae of intercalary oogonia (Text-fig. 2D). Diameter range of lateral oogonia (17) 25–29 (40) μ. Terminal and intercalary oogonia similar but larger, dimensions averaging approximately 31×66 μ, less numerous (approximately 10% of all oogonia). As many as 10 intercalary oogonia may occur in a chain. *Antheridia* androgynous (Text-figs. 1G, 2A, B, C, D, G) or semi-hypogynous (Text-figs. 1H, 2F, L) rarely more than one to an oogonium. Antheridial cells clavate, laterally appressed to the oogonium wall. Fertilization tubes present, but difficult to see (Pl. 2, fig. 5; Text-fig. 2L). Oospheres maturing. *Oospores* spherical or irregular, filling oogonium, usually single (less than 2% of oogonia with two or more, and these oogonia usually intercalary), subcentric, the oil droplets often incompletely surrounding the cytoplasm. Range of oospore dimensions: (*a*) those in lateral oogonia (10) 14–17 (22) μ diam.; (*b*) in terminal and intercalary oogonia averaging approximately 16–37 μ diam., often compound. Germination of oospores has been observed. Habitat: soil, damp grassland, Bedgebury Pinetum, Kent, England. Type specimens and culture deposited in the Botany Department, Queen Mary College, University of London.

This species has been found in over 1000 rough cultures from soil of various types and pH at different seasons of the year from five localities in south-east England. Numerous experiments with varying pH, temperature, and liquid nutrients have been performed without success to induce zoosporangial formation. The fungus grows equally well on meal worms and hemp seeds but less well on a keratin substrate.

The structure of the sexual organs distinguishes this species as a member of the Saprolegniales; the absence of constrictions in the hyphae and the very wide diameter of the basal regions of the extramatrical hyphae (comparable to certain *Achlya* species) indicate that it is a member of the Saprolegniaceae. Its generic position is somewhat uncertain because of the lack of zoosporangia, but the structure, size and arrangement of the oogonia and oospores are sufficiently distinctive to identify the fungus, and it appears to show greatest affinity to *Aplanopsis*, hitherto monotypic and represented by *A. terrestris* Höhnk (which I have also isolated), in apparently lacking an asexual stage, in the similarity in size of the oospores, in the androgynous antheridia and in the relatively extensive turf (3·8 cm. diam. in *A. terrestris*). It differs from this species in its distinctive arrangement of oogonia, in the ornamentations of the oogonia (*A. terrestris* has almost smooth oogonia), in the difference in oospore structure (*A. terrestris* is described as centric or subcentric, while *A. spinosa* is markedly subcentric) and in the very great difference in diameter of the basal hyphae (those of *A. terrestris* are only 20–30 μ diam.).



Text-fig. 1. *Aplanopsis spinosa* sp.nov. A–D, Germinating oospores; E, two oogonia developing on a lateral oogonial branch from the main oogonial hypha; F–H, oogonia with antheridia. F, Surface view of oogonium with the antheridium lying between the oogonial projections; G, androgynous and H, semi-hypogynous antheridia; I, peg-like structure on a vegetative hypha. B, C and D, scale *b*; all other figures scale *a*.



Text-fig. 2. *Aplanopsis spinosa* sp.nov. A, B, Terminal oogonia with compound oospores and androgynous antheridia, two antheridia from one antheridial branch in A; C, a single intercalary oogonium; D, a chain of three intercalary oogonia, the lowest with a papilla which has been converted to a lateral branch by a cross-septum; E, a lateral oogonium with a terminal apiculus; F–K, lateral oogonia with mature oospores; H, I, J, oogonia with predominantly papillate, tuberculate and mammiform projections, respectively; L, oogonium with an androgynous and a semi-hypogynous antheridium, the androgynous antheridium with a fertilization tube.

I wish to express my gratitude to Mr H. V. Newby for advice in the preparation of this manuscript, and Mr G. A. W. Denny for the Latin diagnosis.

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EXPLANATION OF PLATE 2

Aplanopsis spinosa sp.nov.

Fig. 1. Habit, showing a main oogonial hypha with profuse development of lateral oogonial branches, some of which bear oogonia terminally.

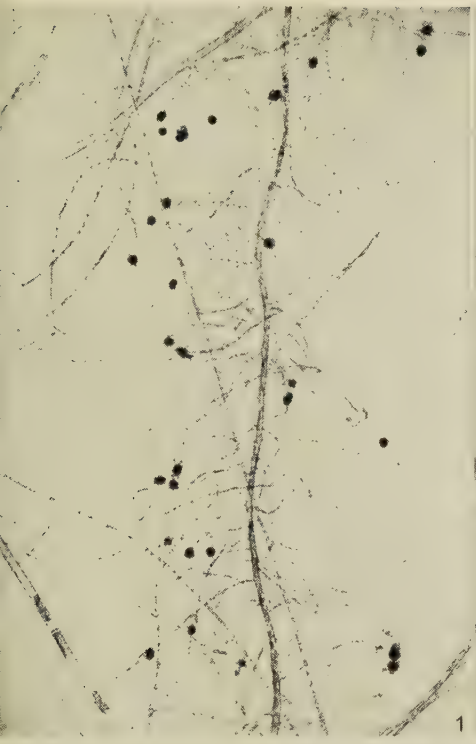
Fig. 2. Main oogonial hypha, with an early stage in the production of lateral oogonial branches showing grouped arrangement.

Fig. 3. Mature oospore in an oogonium with one long tubular projection.

Fig. 4. Main oogonial hypha with lateral oogonial branches and two oogonia, the upper containing a mature oospore in surface view with oil droplets incompletely surrounding the cytoplasm, the lower immature with a closely appressed antheridium.

Fig. 5. Oogonium with an antheridium and fertilization tube (drawn in Text-fig. 2L).

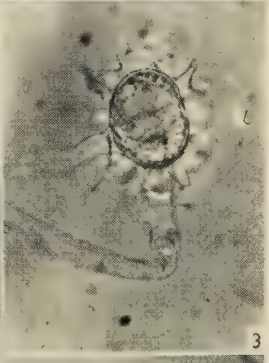
(*Accepted for publication* 5 *March* 1959)

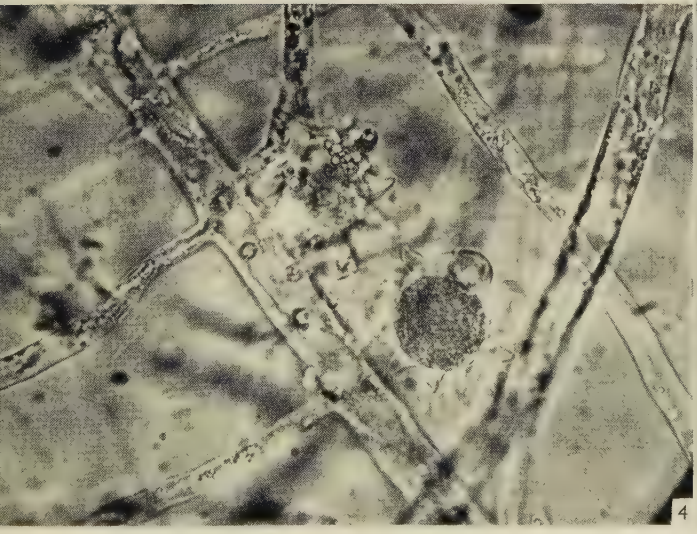


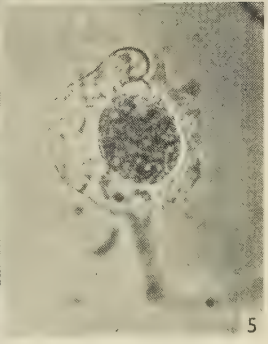
1 500 μ

2 200 μ

3, 4, 5 50 μ







(*Facing p.* 64)

Trans. Brit. mycol. Soc. **43** (1), 65–68 (1960).

PUCCINIA STRIIFORMIS WESTEND. VAR. *DACTYLIDIS* VAR.NOV.

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(With 1 Text-figure)

Puccinia striiformis var. *dactylidis* var.nov. is described and illustrated. It differs from the type variety in its smaller spores, higher temperature optimum for uredospore germination and inability to infect hosts other than *Dactylis*.

Puccinia striiformis Westend. var. **dactylidis** var.nov. (Text-fig. 1)

A typo differt propter uredosporas et teleutosporas minores et pallidiores. Uredosporis 18·5–25 × 15–20·5 μ, teleutosporis 30–49 × 12–22·5 μ.

Hab. in foliis caulibusque *Dactylidis glomeratae* var. *hispanicae* apud Facham, prope Teheran (Iran). Herb. I.M.I. 76632.

Uredosori somewhat paler than those of **P. striiformis* var. *striiformis*. Uredospores 18·5–25 × 15–20·5 (mean 21·5 × 18·7) μ, slightly paler and less regularly echinulate than those of the type variety. Teleutospores 30–49 (mean 39·4) μ long, upper cell 12–22·5 (mean 17·4) μ broad, lower cell 10·5–19·5 (mean 15) μ broad, paler and slightly thinner walled than those of var. *striiformis*, mixed with a proportion of mesospores. Optimum temperature for uredospore germination 21–24° C. On *Dactylis*.

Type specimen on leaves and stems of *D. glomerata* var. *hispanica*, Facham, near Teheran (Iran), coll. G. Viennot-Bourgin, July 1957, Herb. I.M.I. 76632. Other collections examined on *D. glomerata*: Cambridge, 28 Sept. 1945, J. G. Manners; Quy, Cambs, 4 Nov. 1945, J. G. Manners; Ripon, Yorks, 29 Oct. 1946, I. F. Storey; Versailles, near Paris, Sept. 1952, G. Viennot-Bourgin; Forêt d'Écouves, Normandy, 18 Sept. 1952, J. G. Manners (Herb. Southampton U. 159.1); Powerscourt, Co. Wicklow, 13 Sept. 1957, J. G. Manners (Herb. Southampton U. 159.3).

The Facham collection has been selected as the type, since it is the only one, of those examined, with abundant teleutospores. The Versailles collection contains a few teleutospores, but teleutosori are lacking in the British material seen, and do not appear to have been recorded from Britain. The spore dimensions of European material are slightly smaller than those of the type specimen (Table 1). Uredospore dimensions quoted by other authors vary slightly (Table 2), but there is agreement that the uredospores

* *P. striiformis* is commonly known as *P. glumarum* (Schm.) Erikss. & Henn., but this name is invalid (Hylander, Jørstad & Nannfeldt, 1953).

of var. *dactylidis* are smaller than those of var. *striiformis*: the teleutospores are also smaller (Table 1; Fig. 1).

Physiologically, var. *dactylidis* is very distinct from var. *striiformis*. Manners (1950) showed that var. *dactylidis* (then described as race G) differed from all other physiologic races in having a temperature optimum of 21–24° C., as opposed to 10–13° C., and in being strictly confined to *Dactylis*. Consequently race G was not given a number in the 1956 international list of physiologic races of *P. striiformis* (Fuchs, 1956): this list should now be regarded as a list of races of *P. s.* var. *striiformis*.

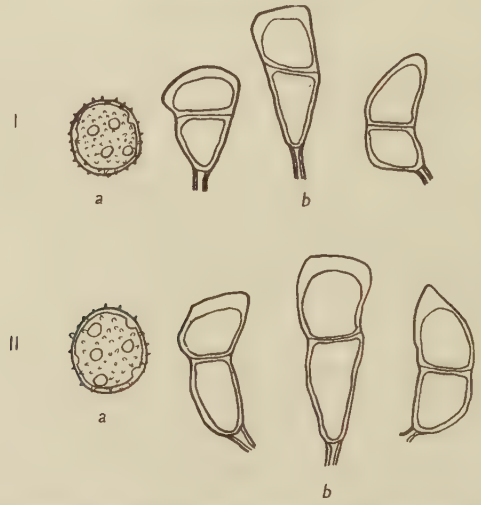

Fig. 1. I. *Puccinia striiformis* var. *dactylidis*: *a*, uredospore; *b*, teleutospores. II. *P. s.* var. *striiformis*: *a*, uredospore; *b*, teleutospores. All in lactophenol, × 500.

Table 1. *Spore dimensions of* Puccinia striiformis *var.* striiformis *and* P. s. *var.* dactylidis

	Teleutospores			Uredospores.
	Mean length (μ)	Mean breadth (μ)		Length and breadth (μ)
		Upper cell	Lower cell	
var. *striiformis*				
Wheat (Herb. Soton U. 159.4)	44·7	17·4	14·8	23·8 × 19·8
var. *dactylidis*				
D. glomerata (Facham)	39·4	17·4	15·0	21·5 × 18·7
D. glomerata (Versailles)	37·9	18·7	16·6	20·7 × 17·6

All spore dimensions are based on the measurement of 100 spores in lactophenol.

Table 2. *Uredospore dimensions* (*previous authors*)

	Length and breadth (μ)	
	P. s. var. *striiformis*	*P. s.* var. *dactylidis*
Viennot-Bourgin (1941)	23·2 × 18·2	20·8 × 17·6
Manners (1950)	24·5 × 18·4	20·2 × 17·2
Kuznetsova (1956)	20·52 × 18·62	18·99 × 16·81

The geographical range of var. *dactylidis* is incompletely known: *P. striiformis*, on *Dactylis* and probably therefore attributable to var. *dactylidis*, has been recorded (mostly as *P. glumarum*) from England (Cudmore, 1932, *in litt.*, Manners, 1950), Wales (Sampson, 1922), Ireland (British Mycological Society, 1958) and, on the Continent, France, (Mayor, 1929; Guyot, 1932; Viennot-Bourgin, 1935, 1941), Holland (Mastenbroek, 1946), Germany (Klebahn, 1898), Scandinavia (Jørstad, 1950; Hylander, Jørstad & Nannfeldt, 1953), Russia (Kuznetsova, 1956) and Iran (Viennot-Bourgin, 1958). There is some evidence that the variety is increasing its range; *Dactylis* is not listed as a British host for *P. striiformis* by Grove (1913), and Jørstad (1950) states that yellow rust first appeared on *D. glomerata* in S. Norway in 1933. The known range of var. *dactylidis* is still much less than that of the species as a whole (Commonwealth Mycological Institute, 1946).

There has been some confusion in the past between *P. striiformis* on *Dactylis* and *P. dactylidina* Bubak (the other rusts on *Dactylis* are easily distinguished from *P. striiformis* by their open teleutosori). A sheet in the Bubak Herbarium bears the two specimens cited by Bubak (1905); one (*leg.* Mühlbuch, 1897), with open teleutosori, is *P. graminis*, as noted by Cummins (1953, annotation to specimen), the other (*leg.* Th. Novak, 1900) has closed teleutosori, as specified in the original description, and spore dimensions corresponding to those given by Bubak (teleutospores 37·5–57·5 (mean 47·0) μ long, upper cell 12–22·5 (mean 17·3) μ broad, lower cell 12–22·5 (mean 15·7) μ broad, uredospores 21–29·5 (mean 24·7) $\mu \times$ 21–26 (mean 22·6) μ. This latter collection should be regarded as the type specimen of *P. dactylidina* Bubak. Bubak (1905) stated that the uredosori of *P. dactylidina* were rusty brown, and assigned the fungus to the *P. dispersa* complex, as did Viennot-Bourgin (1941), who, however, referred to Bubak's fungus as *P. dactylina*. The data from the type specimen, whose spores are larger than those of *P. striiformis*, confirm their view and refute that of Kuznetsova (1956), who considered that *P. dactylidina* was the yellow rust on *Dactylis* now named *P. striiformis* var. *dactylidis*.

My thanks are due to the Director of the Brooklyn Botanic Garden for permission to examine the type specimen of *P. dactylidina*, and to Prof. Viennot-Bourgin, without whose co-operation in supplying me with teleutospore material of *P. striiformis* var. *dactylidis* this investigation would not have been possible.

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(*Accepted for publication* 16 *April* 1959)

Trans. Brit. mycol. Soc. **43** (1), 69–72 (1960).

CALONECTRIA HEDERAE ARNAUD AND ITS *CYLINDROCLADIUM* CONIDIAL STATE

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(With 1 Text-figure)

Cylindrocladium macrosporum var. *hederae*, a parasite of *Hedera helix*, is shown to be the conidial state of *Calonectria hederae*.

Cylindrocladium species have, in recent years, been frequently reported as plant pathogens. Arnaud (1952) described *Cylindrocladium macrosporum* var. *hederae* as causing brown patches and ultimately death of the leaves of *Hedera helix*. In the same paper he also described a *Calonectria* species as *C. hederae*, which, he said, was presumably the perithecial state of the *Cylindrocladium*. The *Calonectria* was only briefly described and as no Latin diagnosis was given the description is invalid. We do not agree with Arnaud who cited *Candelospora citri* Fawcett & Klotz (1937) as a synonym of *Cylindrocladium macrosporum* var. *hederae*. Part of the type material of the former species, which was transferred to *Cylindrocladium* by Boedijn & Reitsma (1950), is in Herb. I.M.I. It has smaller conidia and conidiophores than *C. macrosporum* var. *hederae*.

In November 1958 a *Cylindrocladium* associated with a *Calonectria* was found attacking ivy growing under Sitka spruce at Farnham, Surrey, both the perithecial and conidial states being present in abundance. Neither has been recorded previously in Britain.

Rea & Hawley (1912) described *Candelospora ilicicola* from leaves of *Ilex aquifolium*, Clare Island. Boedijn & Reitsma (1950) transferred it to *Cylindrocladium* and showed it to be the conidial state of a *Calonectria*, which they called *C. ilicicola*. This is the only other 3-septate *Cylindrocladium* recorded in Britain. From Boedijn & Reitsma's description of the perithecia and from an examination of Hawley's material of *Candelospora ilicicola* in the British Museum, *Calonectria ilicicola* is found to be similar to *C. hederae* but has larger ascospores and conidia.

The area where the disease occurred is a moist hollow on a south-facing slope. The spruce was planted in 1927 and was due for a fourth thinning so that at this stage little light reached the forest floor, the vegetation on which was mainly a moss layer and ivy. The ivy formed a fairly continuous mat.

When the disease was first noticed there were many scattered patches up to 5 or 6 yd. diam. in which the ivy was dead. Inspection revealed

abundant perithecia and conidia on leaves and stems. When the site was revisited in April 1959 the patches had not extended appreciably, and there were no signs of the perithecia or conidia. All parts of the ivy were attacked and the fungus could be found on large irregular brown patches on the leaves, along the length of stems and on rootlets. Perithecia were present on stems very close to the junction between living and dead tissue. No attempts were made to prove pathogenicity by inoculation or to investigate the course of infection.

Calonectria hederae Arnaud sp.nov.

Stromata vestigialia, perithecium singulum ferentia. Perithecia ovata, aurantia vel rubra 200–300 μ lata, 300–370 μ alta, ostiolo papilliformi 30–40 μ diam. praedita. Paries perithecii pseudoparenchymaticus. Asci clavati, longe stipitati, 8-spori, 160–180 × 24–40 μ; sporae primo subdistichae, dein irregulariter fasciculatae. Ascosporae hyalinae, cylindraceae vel fusoideae, leves, curvatae vel inaequilaterales, 3-septatae, haud constrictae, 45–65 × 6–8 μ. Conidiophora 350–400 μ longa, basi 6–8 μ prope apicem circa 3 μ lata, superne in vesiculam apicalem subclavatam, 30–40 × 8–10 μ producta; ad circa 50–70 μ alta penicillum lateralem saepius singularem ferentia; ramus primarius 15–25 × 4–5 μ; rami secundarii saepius tres 10–15 × 3–4 μ, quinque 2–4 phialides 12–15 × 5–6 μ apicaliter ferentes. Conidia hyalina, levia, cylindrica, utrinque rotundata, 3-septata, 60–80 × 6–8 μ. Habitat in foliis ad ramis Hederae helicis, Farnham, Surrey, J. S. Murray, 6 Nov. 1958. Typus Herb. I.M.I. 75300.

Description

The *Cylindrocladium* conidial state develops from the sparse mycelium over the surface of the infected stems and leaves of the host. Here the slimy conidia can be seen aggregated into small, pale-coloured cones.

The conidiophores are erect with an upright sterile stipe terminating in an apical vesicle and the conidia are produced from phialides borne on short lateral branches. The stipe arises as a sterile hypha which grows at right angles from the surface of the host. It is 6–8 μ wide at the base and normally forms one to two lateral branches when 50–70 μ long. It then continues its growth up to 400 μ and narrows to about 3 μ just below the apical vesicle. This is thin-walled, cylindrical, but narrowing somewhat towards the tip, and measures 30–40 × 8–10 μ.

The lateral branches, which are the true conidiophores, usually branch again after forming a single cell 15–25 × 4–5 μ long. These cells produce 2–4 somewhat shorter cells 10–15 × 3–4 μ apically and these in turn each bear 2–3 apical phialides. The phialides are doliform, 12–15 × 5–6 μ, and the lateral wall is thickened slightly near the apex to form a collar. The conidia are hyaline, cylindrical, with obtuse ends and with three indistinct transverse septa. They measure 60–80 × 6–8 μ. [Examination of Arnaud's material in Herb. I.M.I. showed only three septate conidia to be present and these measured 63–85 × 6·5–7·5 μ.] The perithecia are scattered to gregarious and develop on the same lesions as the conidia. They appear sessile, but in fact are seated on a small stroma which penetrates the tissues of the host. They are oval to globose, yellowish red to red, with a rough outer wall. They measure 200–300 μ diam. and 300–370 μ high; some lateral collapse occurs on drying. In section the lateral wall is 50–60 μ wide and formed of two regions. The outer is of three to four

layers of globose to hexagonal thin-walled cells 18–40 × 10–16 μ. The inner region, which is only 10–12 μ wide, is formed of three to four layers of elongate compressed cells.

The asci are clavate with a rather pointed and undifferentiated apex and a long stalk. They measure 160–180 × 24–40 μ. In the young ascus the ascospores are distichous, but later they become aggregated in the upper half of the ascus.

The ascospores are hyaline, cylindrical with rounded ends and slightly curved. They have three transverse septa when mature and measure 45–65 × 6–8 μ.

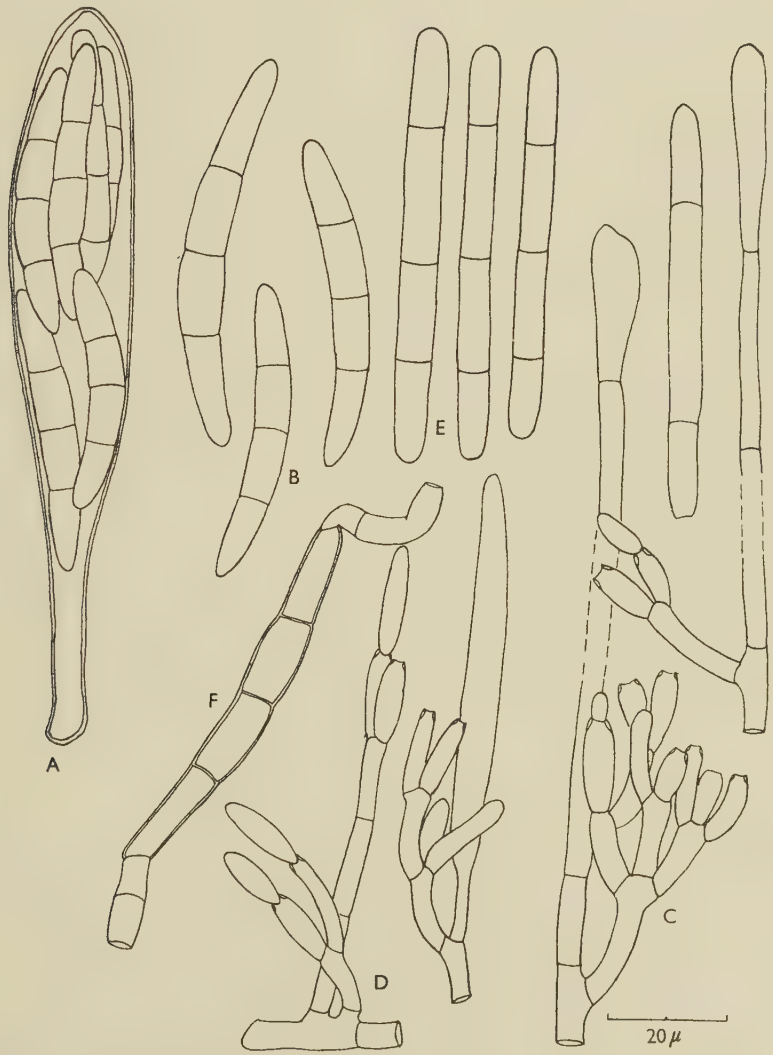

Fig. 1. *Calonectria hederae.* A, mature ascus; B, ascospores; C, conidiophores from host; D, conidiophores from culture; E, mature conidia; F, germinating conidium.

Cultures

The ascospores were isolated on to filtered 2 % maize meal agar and there produced a hyphal primordium from each end. They grew readily on this medium and on 2 % potato dextrose and 2 % potato starch agars. The mycelium was white floccose and covered the surface of an agar test-tube slope in about 7 days. From below, the culture was colourless at first but gradually became reddish brown.

Conidia began to develop in 4–5 days. They were produced both from conidiophores similar to those found on the host and frequently directly from phialides that occurred as termination to short lateral branches developing direct from the mycelium. Sterile stalked appendages also developed independently of the conidiophores and grew to between 150 and 300 μ long with cells 15–35 μ long, 6 μ wide towards the base and 3 μ wide near the apex.

The conidia formed in culture were cylindrical, straight with rounded ends and measured 70–100 × 6·5–8 μ. Two to three transverse septa developed slowly and were rather indistinct.

Our thanks are due to Mr E. W. Mason for correcting the Latin diagnosis.

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(*Accepted for publication* 1 *May* 1959)

Trans. Brit. mycol. Soc. **43** (1), 73–79 (1960).

THE MOVEMENT OF FUNGAL SPORES IN SOIL

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(With 3 Text-figures)

The carriage, by water, of fungal spores in sand and in certain soil horizons was investigated. It was shown that, although such movement can be brought about under experimental conditions, its occurrence in the field is unlikely.

The possibility that the downward movement of fungal spores is a factor concerned in determination of the vertical distribution of soil fungi has been examined by Burges (1950). The data then obtained suggested that spores which have mucilaginous, wettable coats wash downwards readily, while spores with waxy, non-wetting coats remain at the surface. A correlation between spore type and depth at which fungi occurred in soil was also noted, typically surface-living species having spores with waxy walls, whereas those at a greater depth had wettable spores.

During an examination of the microflora of a podsol in Delamere Forest, Cheshire, a large population of *Mucor ramannianus* Möller was found at a depth of 20–30 cm. in the B_1 horizon, or humus pan. This observation was based on results obtained by the dilution-plate method, so that the colonies which developed may have been derived from either mycelium or spores. As most of the previous recorded isolations of *M. ramannianus* were from the litter layer or from root surfaces, and as this fungus has small (2–3 μ) wettable spores, it seemed possible that the colonies which grew from B_1 horizon soil might represent an accumulation of spores which had been washed down from the surface, through the sandy A_1 and A_2 horizons, and filtered out by the much more compacted B_1 horizon. Experiments were therefore carried out to see whether the spores of *M. ramannianus* would wash down, and whether the B_1 horizon would act as a filter-bed and collect the spores.

Spore movement in a sand column

A preliminary investigation of the movement of spores of *M. ramannianus* in sand was carried out in a column similar in design to that described by Burges (1950), but constructed of Perspex instead of glass. The loose cotton-wool plug at the base of the column was supported by a base-plate, which was also fitted with a drainage tube. Even packing was obtained when the column was held vertically and kept full of water whilst the dry sterile sand was poured in steadily. Excess water was drained off before the column was used.

A spore suspension was introduced at the top of the column and followed

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by the required amount of washing water. A standard volume (2 ml.) of spore suspension was used, but the amount of washing water and the time of its application were varied, as indicated in the graphs of results. After the column had drained it was placed in a horizontal position and the front plate removed. To avoid interference from spores which might have travelled more rapidly down the smooth Perspex surface, the outer 1 cm. layer of sand was discarded and 1 g. samples were taken from the centre of the column, at 5 cm. intervals throughout its length. The samples were transferred to flasks of sterile water and dilution counts made, the colonies which developed being counted after 48 hr. incubation. Very few contaminants occurred.

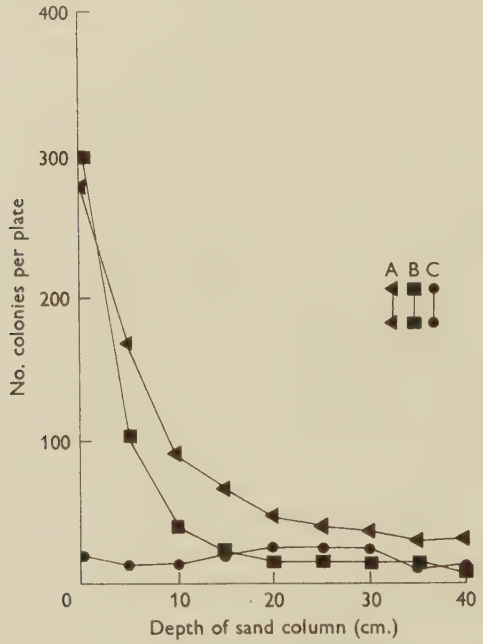

Fig. 1. Distribution of spores in a sand column after the following treatments: *A*, 100 ml. water added *after* the spore suspension had drained away; *B*, 500 ml. water added *after* the spore suspension had drained away; *C*, 500 ml. water added *before* the spore suspension had drained away.

The results (Fig. 1, curve *A*) of an experiment in which 100 ml. of washing water were added showed that the spores of *M. ramannianus* can be washed down through sand. However, the distribution of the spores within the column was unlike that obtained by Burges for small wettable spores, but closely resembled his results for spores with dry walls, e.g. those of Penicillia. Neither filtration of the spore suspension, to remove fragments of mycelium which might have lodged at the surface, nor a fivefold increase in the volume of washing water (Fig. 1, curve *B*), altered the pattern of spore distribution. The surface maximum was finally eliminated when care was taken to begin adding the washing water before the spore

suspension had drained away, thus maintaining waterlogged conditions throughout the washing period (Fig. 1, curve *C*). Although the standard spore suspension had been used in this experiment, the final number of colonies was so much less than in previous experiments that it seemed possible that most of the spores had been washed out of the column. The movement of water down the column, in relation to spore movement, was therefore examined.

A column prepared in the usual way was placed in a dark room and illuminated with ultra-violet light. In place of spore suspension a few ml. of a strong alcoholic solution of eosin were poured on to the top of the drained column. Water was then added in sufficient quantity to keep the

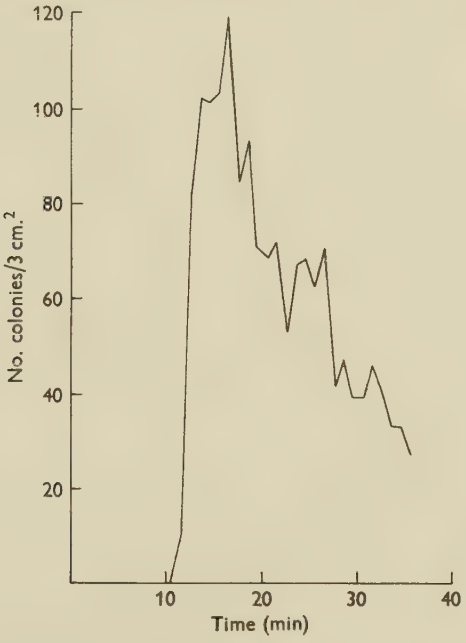

Fig. 2. Results of an experiment in which the drops from the base of the column were caught and plated out.

top of the sand continuously waterlogged. The passage down the column of the brightly fluorescent band of eosin was easily followed; 15 min. after the eosin had been introduced, and when 150 ml. water had been added, the dye reached the base of the column and began to drip out. The first faintly fluorescent drops fell after 12 min. and the main band of eosin after a further 3 min.

The same column of sand was carefully washed and drained, and then a spore suspension, followed immediately by washing water, was introduced. Drops of water began to fall from the base of the column $2\frac{1}{2}$ min. after the water was added. The first drop, and then single drops taken at 1 min. intervals, were dispersed in tubes of nutrient agar and plates poured. As the total number of colonies per plate was very large, the results in Fig. 2

are based on number of colonies in three random 1 cm. squares. The time at which the first spores were recorded—12½ min.—corresponded with the time at which the first fluorescent drops appeared and, similarly, the maximum number of spores were found during the same period as the main band of eosin dripped out, i.e. 14½–18½ min. after addition of the washing water. It was then clear that, under certain conditions, spores moved at the same rate as the water-front. As an eosin-marked water-front was moved through the column by 150 ml. water, the addition of 500 ml. must have removed most of the spore suspension (Fig. 1, curve *C*). It was anticipated that if less than 150 ml. were used the spore maximum would appear in the column, and this was subsequently confirmed (Fig. 3, curve *A*).

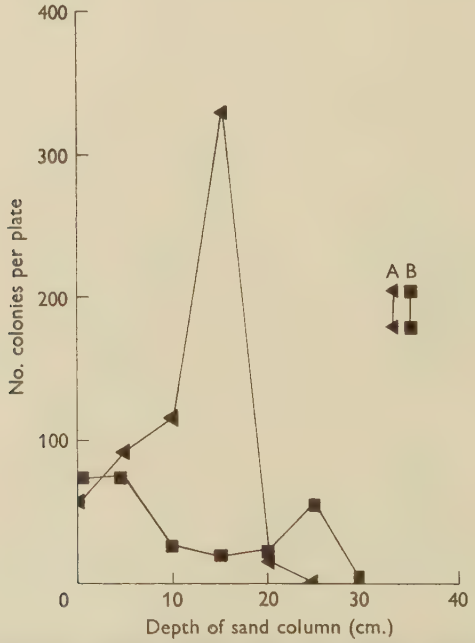

Fig. 3. Distribution of spores in a sand column after the following treatments: *A*, 45 ml. water added before the spore suspension had drained away; *B*, spore suspension added, drained for 1 min., 60 ml. water added.

Further evidence in support of drainage as the cause of the retention of spores in the preliminary experiments was obtained when controlled periods of washing and draining gave rise to spore maxima at predicted levels in the column (Fig. 3, curve *B*).

Spore movement in soil of the Delamere Forest podsol

Attempts to investigate the movement of spores down a column containing a completely reconstructed podsol profile were unsuccessful, for the very fine mineral particles and organic material in the soil prevented even

packing. Each horizon was examined separately and the depths of the soil columns were made to equal the thickness, in the profile, of the horizon under examination. The spore suspension was introduced in the same way as for the sand-column experiments but, owing to the much slower rate of percolation in soil, the washing water had to be added over a 5 hr. period as a slow drip from an aspirator. The position of the spores was determined by isolation on dilution plates. It was shown that spores of *M. ramannianus* could be washed through soil of the A_1 and A_2 horizons, but that B_1 horizon soil prevented this movement.

Spore retention in sand

An examination of the results of the spore-washing experiments showed that sand which had been allowed to drain while it contained the spore suspension retained seventeen times as many spores as it did under water-logged conditions. The reason for such a retention of spores, despite prolonged washing, is not clear, but it is possible that it may be due to the strong adhesion of the spores to the sand grains. As evidence that small bodies, such as bacterial spores, carry electrical charges when they are suspended in an ionizing medium was already available (Abramson, Moyer & Gorin, 1942), it was considered possible that a charge on the spores of *M. ramannianus* might be responsible for their retention after drainage.

The measurement of the charge on the spores was made in a micro-electrophoresis apparatus, the details of which are given by Douglas (1947). The electrophoresis cell was attached to the stage of a microscope used in the horizontal position, and fitted with × 10 eyepiece and × 40 objective. A spore suspension was obtained by washing the surface of a culture of *M. ramannianus*, grown in pans on 2 % malt agar, with a buffer solution. The suspension was concentrated by centrifugation until it contained 10^6 spores/ml. Michaelis's buffer (sodium veronal + acetate + chloride + hydrochloric acid), which is effective over the range pH 2·5–9·5, was used throughout, to prevent errors which might have resulted from changes in the buffering ions. It was intended to use the buffer at the same ionic strength as the soil solution, i.e. 0·0005 N, but, as the buffer is no longer effective at this dilution, a concentration of 0·005 N was used. All measurements were made in a working cabinet thermostatically controlled at 25° C.

The cell was filled with the spore suspension, allowed to equilibrate until the spores showed no directional movement, and then a current was passed. Over the pH range of the podsol, 3·4–4·7, the spores moved towards the anode, and over the whole range of the buffer no change in the direction of movement was recorded.

These results indicated that, at the pH of the Delamere podsol, the spores of *M. ramannianus* would be negatively charged with respect to the liquid in which they were suspended.

Discussion

It is clear from the experimental results that, under certain conditions, sand and the soil of the A_1 and A_2 horizons of the Delamere podsol will permit the passage of water-borne spores of *M. ramannianus* but that B_1 horizon soil restricts this movement. This evidence, at first sight, appears to support the suggestion that the B_1 horizon could act as a filter bed for spores washed down from colonies at the soil surface, but a closer examination modifies this impression.

The addition of 45 ml. washing water to a column of sand moved the spores 15 cm. The cross-sectional area of the sand surface was 10·5 cm.^{2}, therefore this amount of water would be equivalent to 40 mm. rainfall. In order to maintain waterlogged conditions this water was added in 7–8 min., so that the rate of addition corresponded to a rainfall rate of 300 mm./hr. A little over 50 mm./hr. is the greatest rate allowed for on British instruments and such showers are rare, short and very local. The mean monthly rainfall at Delamere (59·07 mm., Bidston Observatory), falling at maximum spore moving efficiency, might move spores 15–20 cm. It would therefore require 2 months' rainfall, at maximum spore-moving efficiency, to transport spores from the soil surface to the B_1 horizon. Even allowing for the smaller amount of water needed to saturate the finer sandy soil at Delamere, the amount and rate of rainfall required is greater than can reasonably be expected, for a rainfall of more than 50 mm./hr. occurs, on the average, only once in 106 years.

It seems fair to conclude therefore that, although it is possible for spores to be washed through the A horizons, the conditions required to facilitate a mass movement of spores to the B_1 horizon are unlikely to occur, and even less likely to occur at sufficiently regular intervals to maintain large numbers of spores in the B_1 horizon throughout the year. If water movement does play any part in the vertical movement of spores, it is likely to be over short distances only.

In connexion with the retention of spores after drainage, it has been shown that the spores of *M. ramannianus* are negatively charged over a wide pH range. Under field conditions sand grains are also negatively charged, so that direct attraction would not occur and, as free multivalent cations which might act as linkages are not available, it seems improbable that the charge carried by the spores is responsible for the retention.

The wettable nature of the spores of *M. ramannianus* suggests that they have a mucilaginous coating. This surface itself may provide an adhesive mechanism for when, on drainage, the spores are brought into close contact with the sand grains by the retreating menisci, there is probably some degree of wetting of the sand surfaces by the mucilaginous walls of the spores. This type of wetting can result in very firm adhesion and, once formed, such bonds are broken only with difficulty. Spores so attached would not be easily dislodged even when the inter-grain spaces were again filled with moving water.

In the light of this new evidence it appears that Burges's apprehension, that the water carriage of wettable fungal spores in soil might give rise to a false picture of the vertical distribution of soil fungi, is unfounded.

The conditions needed to bring about the movement envisaged are very specialized and seem likely to occur in nature only exceptionally. On the contrary, short periods of waterlogging followed by drainage, which must occur frequently in surface soil, may have considerable ecological significance. It has been shown that these conditions allow most of the available spores to be carried a short distance only and subsequently the spores are firmly re-deposited. In the soil the substrates available to fungi are most abundant in the top 5–10 cm. and are usually supplemented from above; furthermore, there is evidence to suggest that the roots which penetrate more deeply carry their surface flora with them (Harley & Waid, 1955; Robertson, 1954), and consequently do not provide open sites for colonization. Small movements therefore are likely to be of value within the dimensions of the soil micro-habitats, whereas large-scale vertical distribution would appear to confer little advantage.

This work was carried out during the tenure of an Isaac Roberts postgraduate studentship in the University of Liverpool, and formed part of a thesis submitted for the degree of Ph.D. I should like to acknowledge the help and encouragement of Prof. N. A. Burges and Dr H. W. Douglas.

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(*Accepted for publication* 27 *April* 1959)

Trans. Brit. mycol. Soc. **43** (1), 80–84 (1960).

RAMULISPORA SORGHICOLA SP.NOV.

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(With 6 Text-figures)

Ramulispora sorghicola, which is the cause of a common leaf disease of sorghum in Nigeria, is described and illustrated and compared with *Gloeocercospora sorghi* and *Ramulispora sorghi*.

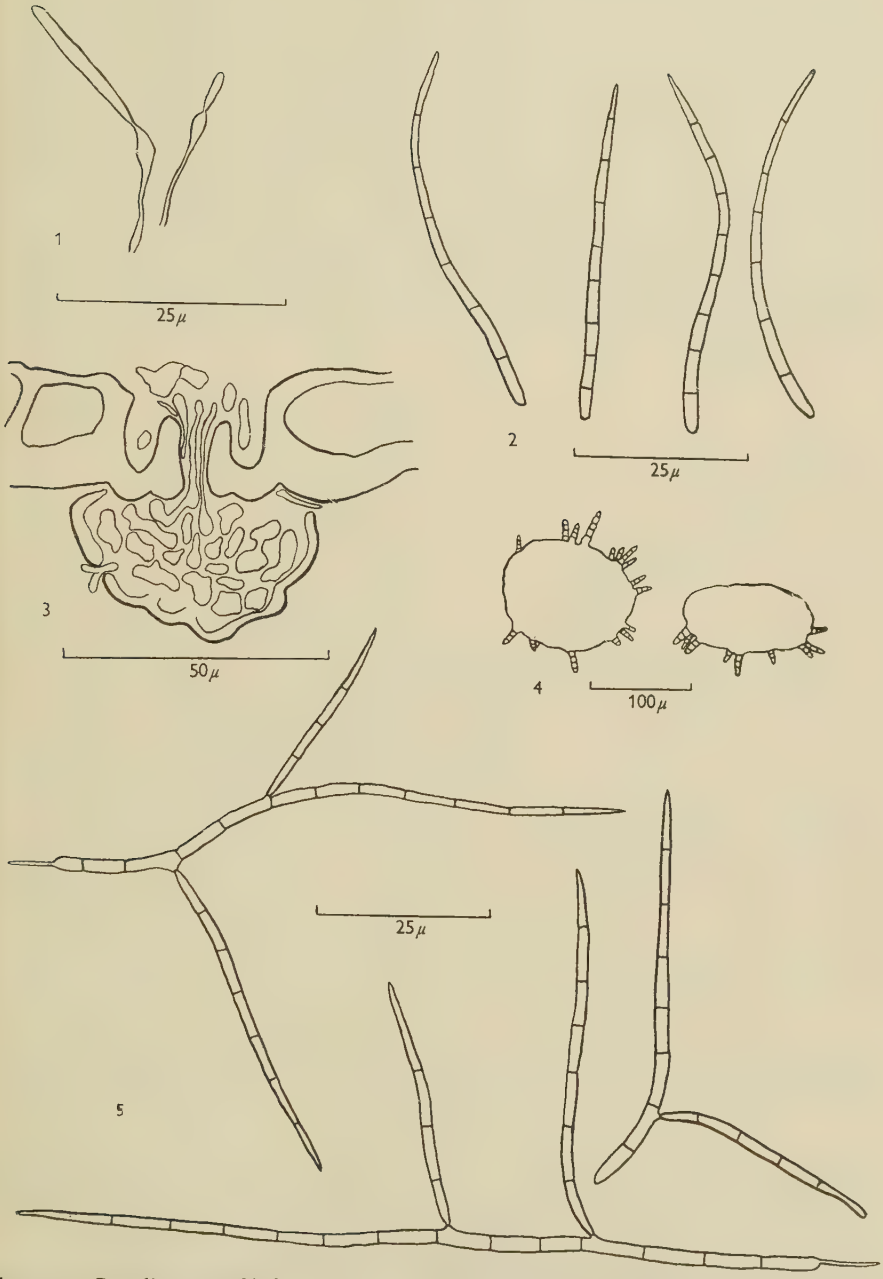
Ramulispora sorghicola sp.nov.

Maculae ellipticae, usque ad 7 mm. longae et 3 mm. latae, plerumque 3–4 × 1·5 mm., vel confluendo irregulares, rosaceo-griseae, interdum stramineae, margine atrorubro conspicuo usque ad 1 mm. lato cinctae. Mycelium in mesophyllo folii immersum, inter cellulas penetrans, ex hyphis hyalinis, septatis, ramosis, 2·5–4 μ latis compositum, in cameris substomatalibus nodosum. Conidiophora (phialophora) per stoma ex mycelio substomatali nodoso oriunda, usque ad 20 in fasciculum aggregata, subclavata, hyalina, continua vel raro septata, 9–12 (20) μ longa, 1·5–4 μ lata, saepius vix prominentia. Conidia numerosissima, ex apice conidiophori in pustulas glutinosas singulariter producta, filiformia, versus apicem subacutum attenuata, recta vel curvata, hyalina, 2–10-septata, 20–75 μ longa, 1·1–2·4 μ lata. Sclerotia ex mycelio per stomata erumpenti saepe producta, in quaque macula usque ad 20 (raro plura), superficialia, dispersa, plerumque hypophylla, subhemisphaerica vel depresso-globosa, atra, 80–190 μ diam., 50–70 μ alt., setis usque ad 30, robustis, rectis, simplicibus, pellucide brunneis, 1–5-septatis, versus apicem obtusum gradatim attenuatis, usque as 40 μ longis, ad basim 9 μ latis, ornata.

In cultura (in 'cornmeal agar') mycelium lentissime crescens, primo album, denique atroviride, conidia in pustulas glutinosas numerosas carnosas nitentes efficiens. Conidia filiformia, hyalina, curvata, ad apicem attenuata, 5–14 septata, 52–126 μ longa, 1·3–2·6 μ lata, ramulis lateralibus usque ad 3 (saepius 2), 1–10-septatis, 10–84 μ longis, 0·8–2 μ latis, etiam cilio basali usque ad 15 μ longo, praedita.

Habitat in foliis Sorghi vulgaris Pers., Samaru, Zaria Province, Northern Nigeria, leg. E. Harris, 27. ix. 1958, Herb. I.M.I. No. 76008 typus.

Leaf spots appearing at first as small water-soaked areas with an indistinct brick-red margin, later elliptical, up to 7 × 3 mm. (mostly 3–4 × 1·5 mm.), becoming irregular by confluence, limited by the veins, with a conspicuous dark red margin (tan in some varieties of sorghum) up to 1 mm. wide and pinkish grey to straw-coloured necrotic centre sometimes bearing scattered black sclerotia. Mycelium in the mesophyll, intercellular, forming knots in the substomatal cavities, composed of hyaline, smooth-walled, septate, branched 2·5–4 μ-wide hyphae. *Conidiophores* (phialides) very small, arising from the knots of mycelium in the substomatal cavities in fascicles of up to 20, passing through the stomata but usually scarcely protruding, subclavate, hyaline, rarely septate, about 9–12 μ long (or up to about 20 μ on old material) and 1·5–4 μ broad at the widest part. *Conidia* formed one at a time from the apex of the conidiophores, very numerous, particularly on the underside of the leaf, radiating from the



Figs. 1–5. *Ramulispora sorghicola* sp.nov. 1. Conidiophores with conidia emerging. 2. Conidia from leaf spots. 3. Developing sclerotium from a transverse section of the leaf. 4. Mature sclerotia from above and from the side. 5. Branched conidia from culture on cornmeal agar.

stomata or agglutinated in cone-shaped, flesh-coloured, gelatinous masses, filiform, straight or curved, hyaline, tapering towards the subacute apex, 2–10-septate and 20–75 × 1·1–2·4 μ. *Sclerotia* formed on some spots, late in the season, by mycelium growing out from the stomata, up to 20 on each spot (more in some collections), superficial, scattered, mostly on the underside of the leaf, subhemispherical to flattened globose, black, 80–190 μ diam. and 50–70 μ high, bearing on the free surface up to 30 stout, straight, simple, 1–5-septate, clear brown setae up to 40 μ long and 9 μ wide at the base tapering evenly to the obtuse apex.

Cultures on cornmeal agar very slow growing, mycelium white becoming dark green and bearing numerous glistening, cone-shaped, flesh-coloured masses of conidia which are filiform, hyaline, curved, tapering towards

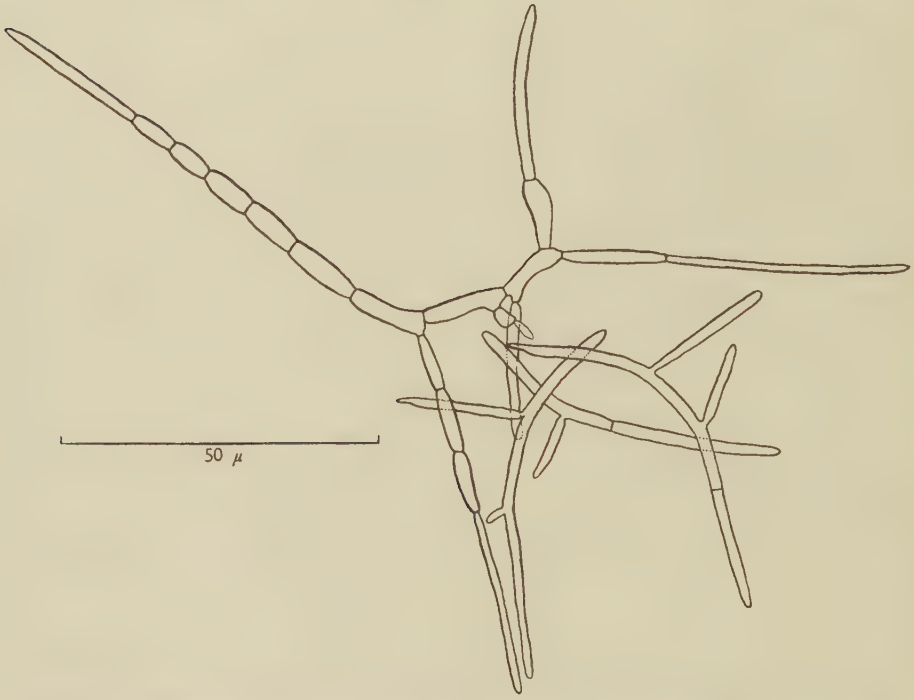


Fig. 6. *Ramulispora sorghicola* sp.nov. Branched conidium germinating on culture medium to produce secondary conidia.

the apex, sometimes with a basal cilium up to 15 μ long, 5–14-septate, 52–126 × 1·3–2·6 μ and bearing up to 3 (usually 2) 1–10-septate lateral branches 10–84 × 0·8–2 μ. Conidia germinate in culture to produce numerous secondary conidia and slow-growing mycelium.

On leaves of *Sorghum vulgare* Pers., Samaru, Zaria Province, N. Nigeria, E. Harris, 27 Sept. 1958, Herb. I.M.I. 76008 (type).

Leaf spots on sorghum caused by *Ramulispora sorghicola* are readily confused with those caused by *R. sorghi* (Ell. & Ev.) Olive & Lefebvre

and *Gloeocercospora sorghi* Bain & Edgerton, particularly if the morphology of conidia on the leaves is considered. The fully developed lesions, however, are quite distinct in appearance. *R. sorghi* causes straw-coloured, elongate-elliptical lesions, while those caused by *G. sorghi* are roughly circular or semicircular with conspicuous broad dark red zonations.

Although *R. sorghi* is described (Olive, Lefebvre & Sherwin, 1946) with branched conidia many collections have predominantly simple conidia. This was noted by Saccas (1954), who pointed out that branched conidia are produced on such material in a damp chamber. Collections of *R. sorghi* from Nigeria produced over 60% branched conidia in a damp chamber while *R. sorghicola* under the same conditions produced less than 10%. In culture almost all the conidia are branched in both species; cultures of *R. sorghi*, however, require several weeks for good conidial production (Olive *et al.* 1946), while those of *R. sorghicola* sporulate abundantly during the first few days.

The clearest morphological distinction between the three fungi is provided by the sclerotia, but these are not always present on the leaf spots. Sclerotia of *G. sorghi* are immersed in the necrotic tissue (Bain & Edgerton, 1943), while those of the other two species are superficial. Sclerotia of *R. sorghi* are tuberculate, glabrous and very profuse, giving the disease its common name of sooty stripe, while those of *R. sorghicola* are fewer and bear septate setae on the surface.

R. sorghicola is one of the commonest fungi attacking the leaves of sorghum in Nigeria and occurs on the crop at all stages of growth. In inoculation tests the fungus was strongly pathogenic; leaf spots started to appear on seedlings 5 days after spraying with a conidial suspension. Attacks on sorghum are often heavy but do not seem to cause premature death of the leaves though up to 25% of the leaf area may be affected.

Although the sclerotia will produce conidia on their surface in the same way as those of *R. sorghi* (Olive *et al.* 1946) the fungus is not dependent on these for survival over the dry season, as spots on fragments of leaf which have lain on the ground through the dry season produce masses of conidia after a few days in a damp chamber.

Heavy attacks of *R. sorghicola* occur on plants growing on land near Samaru where sorghum is planted every year, but attacks are lighter on the experimental station where rotation is practised.

Specimens of sorghum filed under *Gloeocercospora* and *Ramulispora* in Herb. I.M.I. were examined; collections with leaf spots apparently caused by *R. sorghicola* are enumerated below:

Locality	Collector	Herb. I.M.I. numbers
Sudan	S. A. J. Tarr	49955, 44380, 67784(b), 44825(b)*
Nyasaland	P. O. Wiehe	41981*, 34939
Ghana	R. I. Leather	71514(a)
Uganda	B. J. T. Baldwin	71771(a)
Haiti	F. Pierre-Louis	59566
E. Pakistan	G. Watts Padwick	3077

The leaf spots on these collections bear numerous unbranched spores. Sclerotia with setae occur in the two collections marked with an asterisk.

I should like to thank the Director and staff of the Commonwealth Mycological Institute for giving me access to the herbarium and providing facilities; I am especially grateful to Mr Deighton for writing the Latin diagnosis and to Dr Ellis for help in describing the fungus.

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(*Accepted for publication* 14 *April* 1959)

Trans. Brit. mycol. Soc. **43** (1), 85–99 (1960).

SUCCESSION OF FUNGI ON DECAYING COCKSFOOT CULMS

III. A COMPARISON OF THE SPORULATION AND GROWTH OF SOME PRIMARY SAPROPHYTES ON STEM, LEAF BLADE AND LEAF SHEATH

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(With 5 Text-figures)

The frequency of fruiting colonies of some primary saprophytic fungi on decaying stems, leaf blades and leaf sheaths of *Dactylis glomerata* is compared for standing and uprooted culms. Comparative studies of spore germination on glass slides and mycelial growth on ground sterilized grass at various relative humidity values have shown that the primary colonizers often show more rapid spore germination, more rapid growth and a better ability to grow at lower relative humidity values than secondary colonizers with which they were compared. Nutritional differences between grass tissues collected from upper and lower internodes throughout the year have been compared by testing the growth rate of the primary colonizers on a medium composed of 3% dried grass in 2% tap-water agar. Evidence is presented that upper internodes may attain a higher nutritional status than lower internodes during the early period of colonization, but this situation may be reversed later. These findings are discussed in relation to the senescence of host tissues and a hypothesis is presented to explain the observed pattern of fruiting of primary saprophytes on decaying grass stems.

Introduction

Webster (1956, 1957) and Hudson & Webster (1958) have described the succession of fungi found on flowering stems of cocksfoot (*Dactylis glomerata*) and couch grass (*Agropyron repens*). On both these grasses there is a striking difference in the capacity of the upper and lower internodes to support sporulation of fungi which colonize early in the succession. For example, *Cladosporium herbarum*, *Epicoccum purpurascens*, *Alternaria tenuis*, *Leptosphaeria microscopica* and *Pleospora vagans* fruit readily on the moribund upper three internodes, but extremely rarely on the lower three internodes. Hudson has shown that some of these fungi can be isolated from the lower internodes, suggesting that they might be present in the mycelial state, but are unable to fruit.

Hudson & Webster (1958) have listed a number of ways in which upper and lower internodes of the grass might differ and which may influence growth and sporulation of fungi. These factors include differences in water content, nutrient levels, host resistance and competition with other

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organisms. Estimations of the water content of upper and lower internodes showed that the upper internodes have a fluctuating water content, whilst the lower internodes have a consistently higher water content. Experiments in which stems were cut and laid on the ground, resulting in a more uniform high water content of all internodes, showed that certain fungi normally limited to basal internodes could, under these conditions, fruit on the upper internodes. Although the water content of the stem segments could thus be demonstrated to be an important factor controlling fruiting of certain fungi, this factor alone cannot explain completely the differences in distribution of the fungi studied, and in this paper some additional factors have been analysed further for cocksfoot. In section I, further information obtained by comparing the colonization of leaf blades with stem and sheath segments is presented; section II describes experiments comparing growth and germination of various fungi at different humidities. In section III, the results of investigations into the changing nutritional status of the grass are presented.

I. Comparison of colonization of leaf blade, sheath and stem

In the original investigations by Webster (1956), the detailed colonization of the leaf blades was not studied because of the difficulty of obtaining collections of upper leaf blades, which are frequently broken off by wind action. In this investigation the colonization of leaf blades has been followed, and this has made possible a comparison of the sporulation of the primary colonizers on stem, blade and sheath, which sheds further light on the problem.

Monthly samples of ten culms were collected from a plot of cocksfoot growing at the Botany Department Experimental Garden, Taptonville Road, Sheffield 10, from June 1955 to July 1956. The cocksfoot tussocks were part of a clone derived from a single seedling from seed collected at Fulwood, near Sheffield. The plants were propagated by repeated splitting and planted about 50 cm. apart in rows. The stems were incubated for 2 days in damp chambers (for details of technique see Webster, 1956) and the presence of sporulating colonies of fungi was recorded after scrutinizing the culms under a dissecting microscope. The convention used in labelling the internodes follows that used earlier by Webster (1956), the upper internode being no. VI. Where possible the leaf sheaths and blades from the base of the stem were also examined, and these were numbered −I to −VI. Collapse of the culms was prevented by supporting them with a bamboo cane and a loop of wire, and this also helped to prevent loss of upper leaf blades through wind action. During July 1955 and January 1956, a large number of culms was uprooted and laid between the rows of tussocks. The sporulation of fungi on these laid-down culms was compared with that on upright culms.

In representing the frequency of sporulation, separate diagrams have been used for stem, blade and sheath, with histograms showing presence of sporulating colonies with a maximum frequency of ten. Some of the samples had fewer than ten blades or sheaths for a given level, due to detachment or decay, but in calculating the frequency, the values have

been corrected for the smaller number. It should be noted that the internodes corresponding to leaves −VI to −I are very closely inserted, and no attempt has been made to study the sporulation of fungi on the stem segments in this region. The number of leaf sheaths and leaf blades available for examination in the region −VI to −I was very small after January 1956.

Before discussing the results obtained, some physical characteristics of the stem, blade and sheath should first be mentioned. The amount of tissue present in each varies with position, but as shown in Table 1 the dry weight of the stem segments exceeds that of the sheath and blade.

Table 1. *Mean dry weight (mg.) of different portions of the shoot of* Dactylis glomerata, 19 *July* 1955

Internode number	Stem	Blade	Sheath
VI	164	32	75
V	184	45	81
IV	196	45	77
III	189	41	61
II	145	24	40
I	131	19	25
−I	—	13	9
−II	—	19	10
−III	—	20	14
−IV	—	24	18
−V	—	18	18

Table 2. *Water content (% dry wt.) of different portions of the shoot of* Dactylis glomerata

	10 June 1955		19 July 1955			8 Aug. 1955			28 Nov. 1955	
Internode	Bl	Sh	Bl	Sh	St	Bl	Sh	St	Bl	Sh
VI	—	—	137	174	106	94	116	20·8	9·2	16·0
V	—	—	160	188	182	46	128	23·2	10·5	16·7
IV	—	—	93	189	252	28	86	26·5	8·2	16·2
III	—	—	5·2	146	267	13	18·1	24·0	13·7	17·0
II	11·8	332	2·2	167	173	8·3	16·5	62·4	27·0	33·0
I	16·4	432	22·3	250	—	—	26·2	222	—	42·0
−I	25·6	423	112	118	—	—	20·0	—	—	—
−II	21·1	310	54	123	—	12·5	16·7	—	—	—
−III	32·1	363	54	154	—	13·5	33	—	—	—
−IV	16·3	304	40	123	—	67	30·7	—	—	—
−V	20·4	322	14·4	237	—	—	44·5	—	—	—
−VI	21·8	265	—	26·6	—	—	—	—	—	—

The ratios of dry weights for blade, sheath and stem are 1 : 1·7 : 5·1. These differences mean that the blade has the least reserve of nutrients, and might be expected to be depleted more quickly than the other portions. The water content of the different portions also differs widely. The blade projecting laterally from the stem is subjected to greater desiccation than the sheath which clasps the stem. Thus the water content of the blade fluctuates, but in dry weather falls to a low value, especially at the upper levels. Below, the fluctuation is less marked, because the blades are surrounded by the crowded leaves of the tussock. A few estimations of the water content of standing stems is shown in Table 2. The data show that the

water content of the leaf blades is often low, and values are consistently lower than the values for leaf sheath and stem. As noted previously (Webster, 1956), the water content of the upper internodes may fall to a low value within 6 months of flowering.

The frequency of sporulating colonies of four primary saprophytes, *Leptosphaeria microscopica*, *Pleospora vagans*, *Cladosporium herbarum* and *Epicoc-*

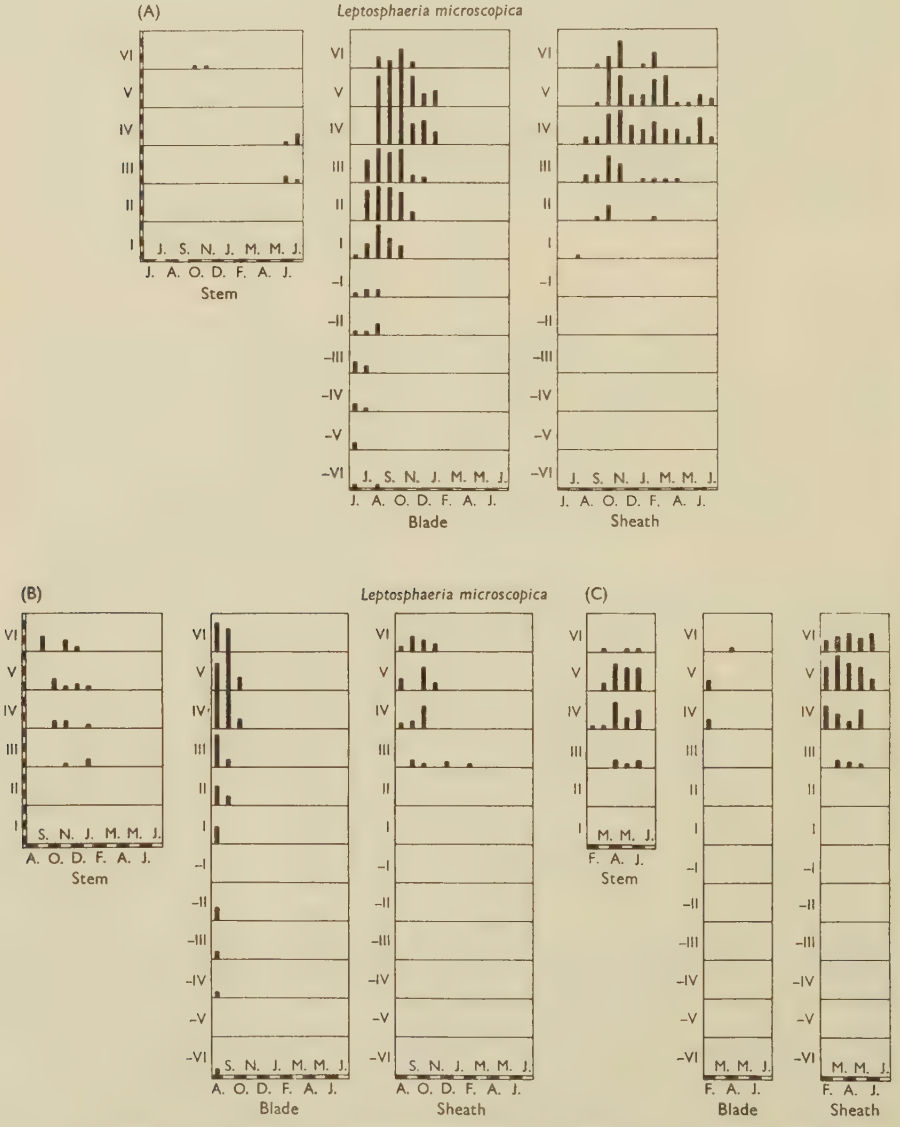

Fig. 1. Frequency of perithecia of *Leptosphaeria microscopica* on segments of cocksfoot stems from monthly samples of ten stems. The numbers refer to the internodes. A, Occurrence on standing stems; B, occurrence on stems uprooted in July; C, occurrence on stems uprooted in January.

cum purpurascens is shown in Figs. 1–4. *Alternaria tenuis*, which in earlier studies has been found to have a similar distribution, was infrequent in this locality. The frequency on upright culms is shown in the first

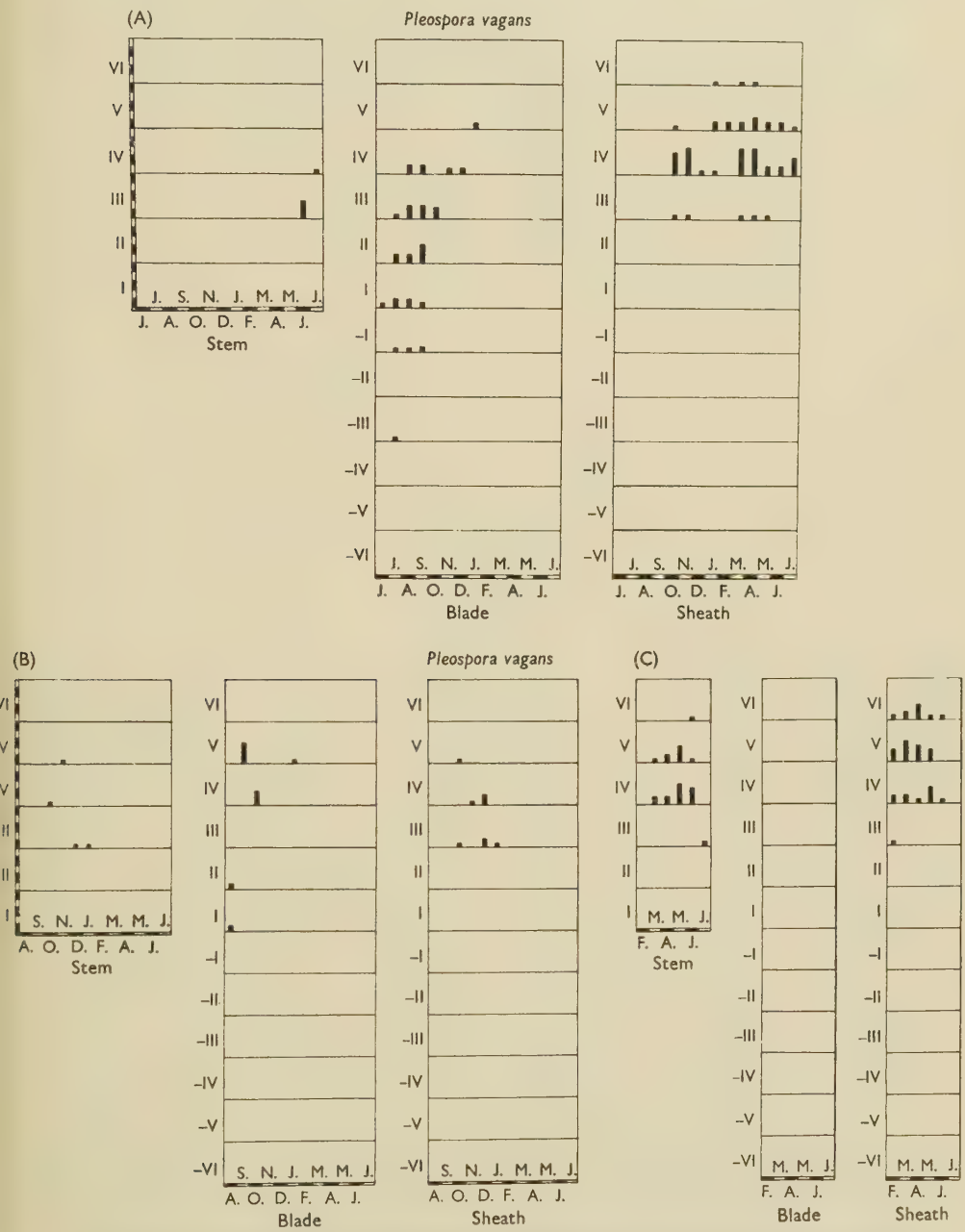

Fig. 2. Frequency of perithecia of *Pleospora vagans* on segments of cocksfoot stems from monthly samples of ten stems. Labelling as in Fig. 1.

figure (A), and is compared in (B) with the frequency on culms laid on the ground in July, and in (C) with culms laid on the ground in January.

Fig. 3. Frequency of *Cladosporium herbarum* on segments of cocksfoot stems from monthly samples of ten stems. Labelling as in Fig. 1.

The diagrams show again the effect noted earlier for stem segments and leaf sheafs—that all these fungi fruit more frequently on the upper internodes, whether on standing culms or on collapsed culms, and fruit very infrequently or not at all on stem segments and sheaths below Internode III. This difference is not found between upper and lower leaf *blades*, however, and the diagrams show clearly that in the early months of the period studied, all these fungi were found sporulating on lower

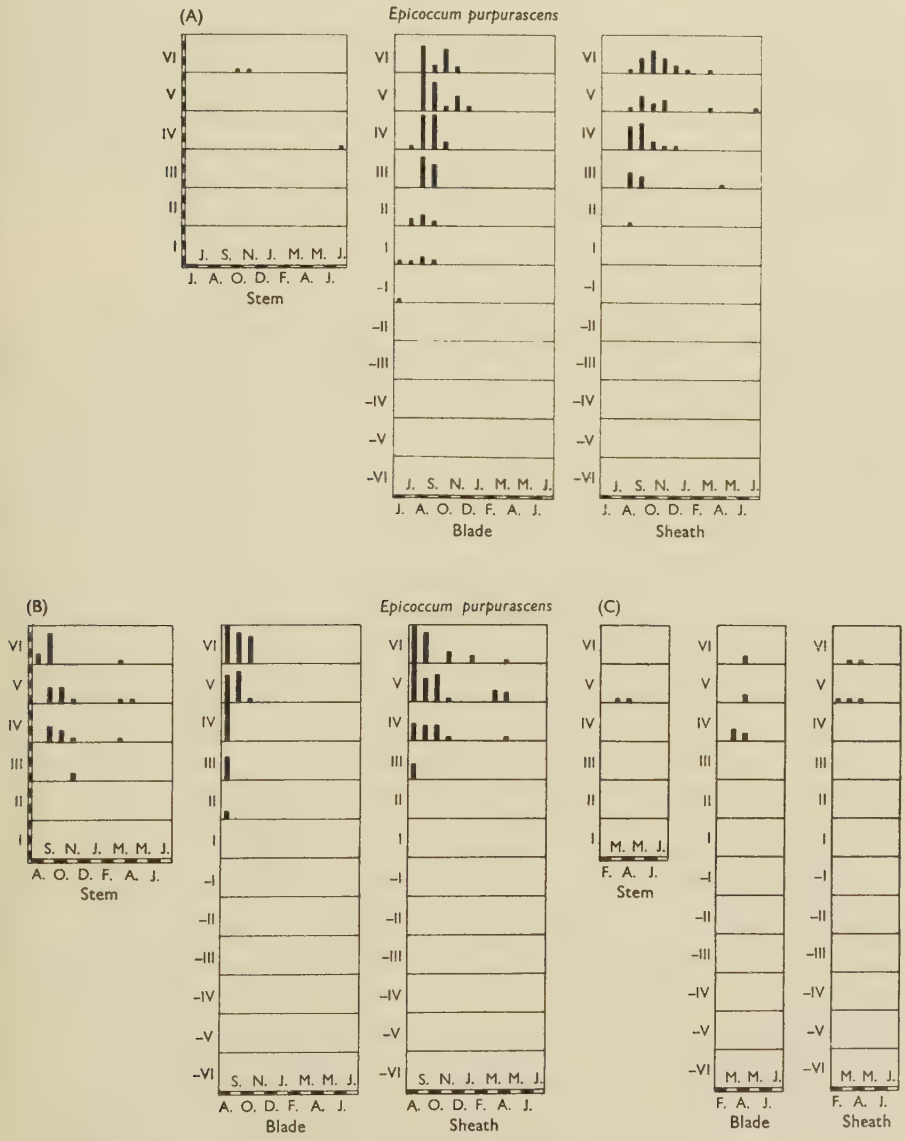

Fig. 4. Frequency of *Epicoccum purpurascens* on segments of cocksfoot stems from monthly samples of ten stems. Labelling as in Fig. 1.

leaf blades (−I to II, and in some cases on still lower leaves). The impression gained from a study of the colonization of these lower leaves is that, in the early stages, it corresponds to the colonization of the upper leaf *sheaths*. During the early stages of colonization the leaf blade remains fairly rigid, standing away from the sheath and the remaining leaves in the tussock. Later, as it becomes moribund, the lower leaf blade tends to collapse downwards and becomes surrounded by other leaves, so that its water content tends to be higher once it has lost rigidity. Colonization by fungi normally associated with damper conditions (e.g. *Dasyscypha* spp., and *Torula herbarum*) then follows, and the primary fungi cease to sporulate. We suggest that competition with other organisms in the damper conditions is the reason why the primary fungi do not continue to fruit for so long on lower leaf blades as on upper leaf blades. The suggestion is supported by the behaviour of these fungi on upper leaf blades of culms laid on the ground in July. Under these conditions the primary fungi virtually disappear within 3 months from upper leaf blades.

II. Growth and germination at varying humidities

The ability of certain primary colonizers to persist on upper internodes, subjected to fluctuating water contents, contrast with those fungi normally confined to the basal internodes, which fruit later in the succession. It has already been shown by Webster (1956, 1957), and by Hudson & Webster (1958), that the stem-base fungi can fruit on upper internodes of cut-down stems, and Webster (1957) has shown that some of them require a higher humidity for germination and germ-tube growth on glass slides than the fungi which persist on upper internodes. However, it was felt desirable to extend the spore germination studies to provide more critical information about the latent period for germination, and to study also the growth of mycelium on grass tissue maintained at different humidity levels. In these experiments, comparison has been made between the following fungi as examples of primary colonizers which persist on upper internodes: *Alternaria tenuis*, *Cladosporium herbarum*, *Epicoccum purpurascens*, *Leptosphaeria microscopica* and *Pleospora vagans*. They have been contrasted with the following stem-base fungi which are not vigorous primary colonizers: *Acrothecium* sp., *Tetraploa aristata* and *Torula herbarum*.

For spore germination experiments, spore suspensions were prepared in 0·1 % agar. The suspension was then spread on slides and allowed to dry, before being placed in humidity chambers and stored at 20° C. The humidity chambers were square-shaped, slide-staining tanks which were well sealed with vaseline when in use. At intervals slides were removed from the tanks and measurements were made of percentage germination and of the average length of thirty germ-tubes taken at random. These data provide information about the growth rate of germ-tubes and the latent period for germination (i.e. the time taken for germ-tubes to appear).

Investigations of the behaviour of the mycelium when subjected to a range of humidities on grass tissue were carried out in half-Petri dishes filled with dried, ground-up grass fortified by 10 ml. of a medium of the following composition: Yeast extract (Difco), 1·5 g.; $MgSO_4\ 7H_2O$,

0·585 g.; NaCl, 0·1 g.; $CaCl_2$, 0·1 g.; $NaNO_3$, 2·12 g.; Glucose, 20 g.; water, 1 l.

The dishes were oven-dried, and sterilized at 20 lb. pressure for 15 min. The sterile dishes were then transferred to larger glass dishes, sealed with a plate glass cover and containing salt solutions to give a range of relative humidities from 100 to 79·5 %. The ground grass was left for a few days to acquire a water content in equilibrium with the surrounding relative humidity. Duplicate dishes for each treatment were then inoculated with small mycelial inocula, taken from stock cultures on oat agar. The ground grass cultures were incubated at 20° C. and, after a suitable period of time, dishes were withdrawn and three measurements of fungal colony diameter were made from each dish. From these measurements, estimates were made of the growth rate at each humidity level studied (Tables 3–5).

Table 3. *Growth rate of mycelium at* 100 % R.H. *(calculated after about* 10 *days in chamber)*

	mm./day	S.D.*
Primary colonizers		
Epicoccum purpurascens	6·45	1·8
Alternaria tenuis	6·41	0·7
Leptosphaeria microscopica	4·30	1·0
Pleospora vagans	4·14	0·7
Cladosporium herbarum	2·96	1·1
Secondary colonizers		
Torula herbarum	1·41	3·1
Tetraploa aristata	3·34	0·7
Acrothecium sp.	1·93	0·01

* S.D. = standard deviation from the mean of six results.

Table 4. *Latent period for germination of spores, and growth rate of germination tubes at* 100 % R.H.

	Latent period (hr.)	Growth rate (μ/hr.)*
Primary colonizers		
Epicoccum purpurascens	0–3	31·6
Alternaria tenuis	3–6	29·1
Leptosphaeria microscopica		
Pycnospores	6–9	17·9
Pleospora vagans		
Pycnospores	0–7	5·9
Ascospores	—	12·1
Cladosporium herbarum	6–12	4·2
Secondary colonizers		
Torula herbarum	12–18	0 (12·0 in liquid water)
Tetraploa aristata	12–18	2·4

* Calculated after about 40 hr. in humidity chamber.

The investigation of mycelial growth of these fungi revealed that, contrary to what might have been supposed, there was very little difference between the capacities of the mycelium of the two groups of fungi to grow at low humidities. After 8 weeks in the humidity chambers, no fungus made growth at a relative humidity below 93 %, but all fungi made growth at 95 % relative humidity. Nor was there any greater capacity among the

primary colonizers, as a group, to make proportionately better growth at lower humidities. The depressing effect of lower humidity brought about specific reactions in the growth rates of individual fungi. However, when mycelial *growth rates* are compared, the two groups of fungi are more or less distinct. The growth of those fungi appearing early in the succession and inhabiting regions of fluctuating relative humidity is, for the most part, considerably faster than that of those appearing later in the succession and confined in their growth to the lower internodes of the grass.

Table 5. *Lowest relative humidity at which germination of spores occurred (in range* 100–89 %)

Primary colonizers	
Epicoccum purpurascens	92 % (no germination at lower humidities after 114 hr.)
Alternaria tenuis	89 %
Leptosphaeria microscopica	
Pycnospores and ascospores	92 % (no germination at lower humidities after 258 hr.)
Pleospora vagans	
Pycnospores	92 % (no germination at lower humidities after 140 hr.)
Ascospores	89 %
Cladosporium herbarum	89 %
Secondary colonizers	
Torula herbarum	Water (no germination at lower humidities after 162 hr.)
Tetraploa aristata	98 % (no germination at lower humidities after 236 hr.)

In Table 3 growth rates at 100 % relative humidity are presented. A similar observation can be made about the growth rates of germination tubes of spores (Table 4).

Further information of interest arising from this study is that spores of the primary colonizers germinate much more rapidly at all humidities (the latent periods at 100 % R.H. are presented in Table 4). Also spores of the primary colonizers germinate at lower relative humidities than those of fungi appearing later in the succession (Table 5).

It is certain that the upper regions of the grass, exposed as they are, are subjected to a fluctuating humidity. At times relative humidities would be very low in these regions whilst at other times, after rain or dew formation, it might be quite high. Whilst it would not be impossible for the spores of those fungi normally associated with the lower internodes of the grass to germinate on upper internodes during more favourable periods, they germinate and grow slowly and might thus fail to take advantage of an ephemeral situation and be unable to establish themselves. In less favourable conditions they might be unable to germinate at all. Spores of the primary colonizers, on the other hand, would have the advantage in that they would germinate in less ideal conditions, growing slowly, flaring up and quickly exploiting, by virtue of their rapid growth, any change towards more favourable humidity conditions. In favourable humidity conditions their spores would germinate rapidly. During dry conditions, it is probable that the primary colonizers persist as pycnidia or perithecia, and develop a further crop of conidia, pycnospores or ascospores during sufficiently prolonged wet conditions.

III. Nutritional differences between leaves from upper and lower internodes

It has been suggested that there might be nutritional explanations of some of the features of the succession of fungi on decaying grass culms, in particular the greater persistence of primary colonizers on upper as compared with lower internodes. To test this suggestion, a comparative analysis of the nutritional status of leaf blades and leaf sheaths from upper and lower internodes, as measured by their ability to support fungal growth, has been made. There is already some evidence of nutritional differences. Richards (1934) writes: 'It is not legitimate to assume that each leaf of barley begins its history with the same constitution and repeats in its turn the same succession of physiological changes at the same rate as every other.... Initial variation from leaf to leaf may be much greater than the change with age in any particular leaf.' Gregory & Richards (1929) and Richards (1932) have in fact shown that there are differences in successive leaves of the same plant in shape, ratio of dry weight to leaf area, ratio of water content to leaf area, water content, rate of respiration, carbon assimilation, colour and chemical composition. There is, however, scant information concerning chemical differences between internodes of grasses subsequent to maturation. Nicol (1936) has reviewed some of the earlier work, most of which is concerned with inorganic constituents. Archbold & Mukerjee (1942) have studied the distribution of carbohydrate in barley, and state that at first the maximum concentration of sugar is at the base of the plant, in leaf sheath and stem. Later, as successive internodes reach maturity, the maximum concentration shifts upwards, so that finally the top internode reaches a higher level, just prior to harvest, than that previously attained at the base, and this polarity persists until after harvest. The percentage differences between upper and lower internodes of the *stem* are small, but in the case of the sheath the carbohydrate content may be up to four times as great at the top as at the base of the plant. The flag leaf sheath contained as much sugar as all the leaves together at their maximum. If similar differences could be demonstrated for *Dactylis glomerata*, they might serve to explain the greater persistence of the primary saprophytes at upper internodes.

The comparison has been based on the determination of colony diameters of fungi grown on a solid medium prepared from pulverized parts of the grass. Collections of 40–80 cocksfoot culms were made, from the clones established in the garden, at monthly intervals (usually on the 25th of each month) from May to December 1957. The culms were air-dried in the laboratory, and then each monthly sample was divided into separate internodes, which were further separated into stem, leaf sheath and leaf blade. For the growth experiments samples of the upper three internodes were pooled, for comparison with pooled samples from the lower internodes. Each batch of material was ground to a fine powder in a laboratory mill. Preliminary experiments to discover a suitable concentration of ground grass for growth in a medium consisting of ground grass, tap water and 2 % agar showed that 3 % grass was the optimum concentration, and this was chosen as a standard in all experiments. Similarly, preliminary

experiments showed that the temperature optimum for growth in many of the fungi was 20° C., and experiments were conducted at this temperature. In preparing the medium a direct weighing method was adopted. The amount of ground grass to give the desired concentration was weighed separately into vials and 15 ml. of hot 2 % tap-water agar added. The vials were then sterilized for 15 min. at 20 lb. pressure. After sterilization the medium was poured into 10 cm. sterile Petri dishes, and agitated gently to ensure even distribution of the ground grass. For inoculation, 4 mm. disks were cut out from the margin of a fungal culture on an identical medium. In most experiments two dishes were inoculated, but in later months, when material was insufficient, sometimes only one dish was possible. The cultures were incubated for 6–10 days, and measurements of three colony diameters were made on each dish, using a travelling microscope.

The results are shown graphically in Fig. 5. In addition to the four fungi of which the distribution has been discussed, two others were included for comparison: *Pleospora infectoria*, one of the known perfect states for the species *Alternaria tenuis* which has a similar distribution to other primary saprophytes, and *Pullularia pullulans*, which has been shown by Hudson & Webster (1958) to be an important primary colonizer of *Agropyron repens*.

The curves for mean colony diameters show that, in all cases during the early months of the period studied, the upper leaf sheath tissues were capable of supporting more rapid growth of the primary fungi than the lower internode tissues; but that in later months the situation was frequently reversed, and the lower internode tissue was superior as a growth medium.

Discussion

The succession of fungi on decaying grass poses an intriguing ecological problem: to explain the strikingly different course of succession followed by upper and lower internodes. In this paper we have attempted to isolate and study quantitatively the effects of nutritional status, water content and humidity. These factors do not, however, operate in isolation, but in conjunction, and against a background of resistance by the host to colonization. Webster (1957) and Hudson & Webster (1958) have emphasized the importance of this effect, which is difficult to study quantitatively. They have pointed out that the upper internodes undergo senescence rapidly after the grass host matures, but that the lower internodes lose resistance to fungal colonization only slowly, frequently remaining green for several months after flowering. The interplay of these three factors, the humidity gradient, the nutritional status of the host tissue, and the rapidity of its senescence, operating through the varying physiological capabilities of the fungal colonizers, may provide a plausible explanation of the observed facts.

Evidence has been presented that the upper leaf sheaths attain a higher concentration of nutrients than the lower internodes. It is probable that the subsequent reverse in nutrient levels is explained by differences in the rate of senescence. The upper internodes, after the flowering of the grass,

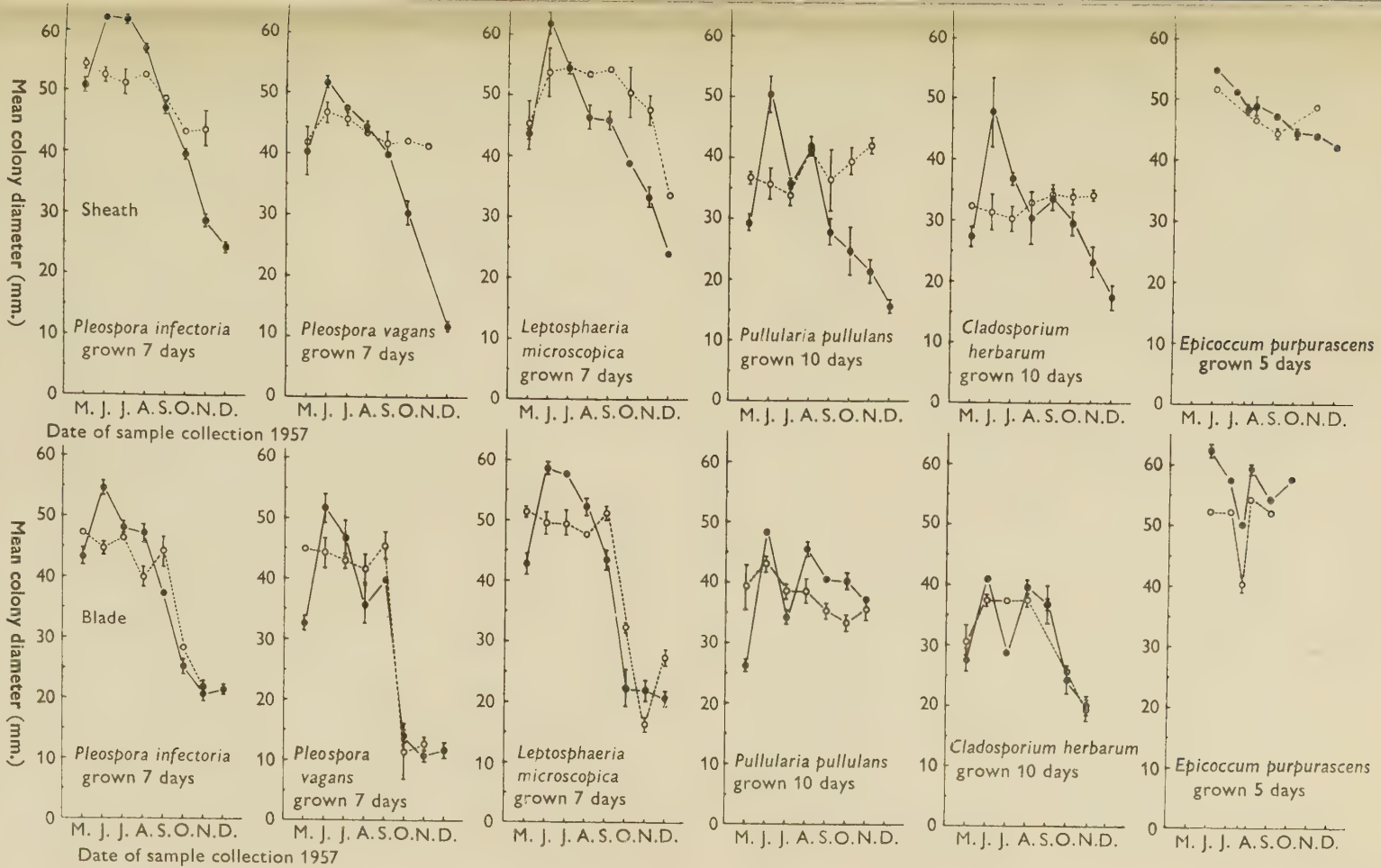
Mean colony diameter (mm.)
Sheath
Pleospora infectoria grown 7 days
Pleospora vagans grown 7 days
Leptosphaeria microscopica grown 7 days
Pullularia pullulans grown 10 days
Cladosporium herbarum grown 10 days
Epicoccum purpurascens grown 5 days
M. J. J. A. S. O. N. D.
Date of sample collection 1957
Blade
Pleospora infectoria grown 7 days
Pleospora vagans grown 7 days
Leptosphaeria microscopica grown 7 days
Pullularia pullulans grown 10 days
Cladosporium herbarum grown 10 days
Epicoccum purpurascens grown 5 days

Fig. 5. Growth rate of some primary saprophytes on 3% ground grass agar, prepared from samples of upper and lower leaf sheaths and leaf blades collected at monthly intervals. ●—●, Growth on upper internode material; ○- - -○, growth on lower internode material.

undergo rapid senescence, accompanied by vigorous sporulation of the primary saprophytes, and this period of senescence is also accompanied by a steep fall in the nutrient status of the upper internodes. By contrast, the lower internodes may remain green for several months (and in some cases give rise to new tillers), that is to say, the rate of senescence is retarded and the rate of decline in nutrient status is less rapid.

If at maturity the upper internodes have a high level of nutrients, which because of rapid senescence of the host tissues are made readily available to vigorous primary colonizers, it seems reasonable to suppose that this would enable the fungi capable of exploiting these tissues by virtue of rapid germination and growth to accumulate reserves of nutrient in their mycelia which might allow them to continue sporulation for a longer period. Below, with an initially lower level of nutrients, senescence is delayed, so that only gradually do the nutrients of these tissues become available to the fungal colonizers. This might result in insufficient nutrient reserves in the mycelia to allow prolonged sporulation.

In the case of leaf blades there is also evidence of a higher nutritional status in the upper internodes, but there is no obvious difference in the rate of senescence between upper and lower internodes; at both levels the rate of senescence of leaf blades is fairly rapid. This is reflected in the slopes of the curves shown in Fig. 5, which are roughly parallel for both upper and lower blade material.

The incidence of fruit bodies of the primary saprophytes is thus greater on tissues showing rapid senescence (e.g. leaf blades, upper leaf sheaths) and is prolonged on those tissues which enjoy a fluctuating water content (especially on upper leaf blades and upper leaf sheaths). The higher incidence of primary saprophytes on upper internode tissues is also correlated with a higher nutrient status of these tissues at the time of colonization.

These conclusions may also apply to fungi other than those considered here. Last (1955) has shown that the numbers of colonies of *Sporobolomyces* per unit area of leaf blade of spring-sown wheat are higher on the upper leaves than on the lower leaves. The values for lower leaves do not exceed 20 cm.^{2}, whilst the values for the upper leaves are frequently more than twice as high. Last has interpreted these results in terms of atmospheric humidity correlated with crop density, but it seems possible that the development of *Sporobolomyces* might be more intense on the upper leaves as a result of a higher nutritional status.

Whitney & Mortimore (1958) have shown that an inhibitory substance extracted from young corn stalks suppresses the growth in culture of *Gibberella zeae* and *Fusarium moniliforme*. Whilst we have no evidence of such inhibitors in the case of cocksfoot, the possibility of their existence should be borne in mind. If such a substance could be shown to be present in higher concentration in the basal internodes of grasses, capable of suppressing the growth of primary colonizers, an alternative explanation of the distribution patterns here observed might be possible.

We are grateful to Miss N. Lomas for technical assistance. One of us (N. J. Dix) is indebted to the D.S.I.R. for a Maintenance Grant.

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(*Accepted for publication* 4 *May* 1959)

Trans. Brit. mycol. Soc. **43** (1), 100–104 (1960).

SOME OBSERVATIONS ON *TRACHYSPHAERA FRUCTIGENA* TABOR & BUNTING, WITH PARTICULAR REFERENCE TO JAMAICAN BANANAS

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(With Plate 3)

Trachysphaera fructigena Tabor & Bunting is recorded for the first time on Jamaican bananas of the Lacatan variety. The fungus was observed occasionally on almost mature fruit, which had been stored for several days inside ripening rooms in England, but not on newly arrived, green fruit. Symptoms of disease are comparable with those often observed on fruit from West Africa. Experimental inoculations showed that the fungus readily infects wounded, immature fruit, often resulting in the appearance of advanced symptoms after 5–7 days storage at 65–75° F. All attempts to infect healthy, unwounded fruit were unsuccessful. Infection of Jamaican fruit was thought to be initiated by spores of *T. fructigena* which had remained inside the ripening rooms after removal of severely infected West African fruit; infection may have occurred through existing skin injuries. Ripening room hygiene is discussed.

Introduction

Trachysphaera fructigena was first described by Tabor & Bunting (1923) as the organism causing mealy-pod disease of cocoa and coffee in the Gold Coast. During recent years the fungus has been recorded on bananas growing in West Africa (Brun & Merny, 1947; Brun, 1954; Thorold, 1956). However, there appears to be no record of the fungus on these hosts in the West Indies.

In the field, *T. fructigena* is reported (Brun, 1954) to produce symptoms similar to those of 'Cigar-end' disease caused by *Verticillium* (*Stachylidium*) *theobromae* (Wardlaw, 1935). Tissues at the blossom-end of the finger* darken and then shrink, so that there is a folding of the skin. Diseased areas become covered with conidiophores, so that the distal end resembles a burnt cigar tip. Brun & Merny (1947), working on Cameroon Gros Michel fruit which had been shipped to Dieppe, observed a new form of fruit-rot caused by *T. fructigena*. Infection was not always restricted to the blossom-end and frequently extended throughout the length of the finger.

The present paper describes observations on *T. fructigena* causing a rot of Jamaican bananas after arrival in the United Kingdom.

* *Note on Terminology.* 'The individual banana fruit may conveniently be described as a "finger", and its short stalk as the "finger-stalk"; where several "finger-stalks" meet to form a "hand", a crescentic "cushion" is formed. These "cushions" project from the "main-stalk" of the whole bunch' (Wardlaw, 1935, p. 408).

Occurrence on Jamaican and other fruit

Although the fungus was often observed at English ports on newly arrived Gros Michel fruit from the British Cameroons and Fernando Po, it was never found on newly arrived Jamaican Gros Michel or Lacatan bananas. However, on three occasions, several bunches of infected Lacatan fruit were discovered inside ripening rooms. This fruit had been stored for 7–9 days, and, in some instances, symptoms of disease were as advanced as those observed on mature Cameroon fruit, which was already infected on arrival in the United Kingdom.

Rotting of Jamaican fruit was rarely of the typical 'Cigar-end' type, and infection occurred more or less at random along the finger. The disease was most frequent on the outer fingers of each hand and least frequent on the less exposed ones; also, in most cases, diseased tissues were centred about some form of old skin injury. Early stages of infection in green fruit were characterized by a buff-yellow or khaki discoloration of the skin, which became slightly depressed. As maturity approached, rotting spread in all directions, the skin turning dark brown or black and becoming thrown into folds. Large numbers of conidia developed on the fruit surface to form a white or pinkish brown mealy incrustation. In the final stage, the complete finger was considerably shrunken and presented a mummified appearance. Initial rotting of the pulp may be described as a dry rot, with a tendency to become tough and fibrous: considerable darkening takes place, particularly along the carpellary axis. In advanced stages of decay, several secondary organisms were associated with *T. fructigena*. These symptoms are similar to those observed by Brun & Merny (1947) on Gros Michel bananas.

Since it was known that the rooms which contained infected Jamaican fruit formerly contained severely infected Cameroon fruit, it seemed reasonable to suspect this as a possible source of inoculum. Conveyor belts, railway trucks or lorries, on which fruit from both regions is transported, were also considered suspect. Further observations and experiments were initiated, therefore, in order to investigate the infection biology of *T. fructigena*.

Experimental infection

Bunches of green Lacatan fruit were sprayed with a suspension of conidia of *T. fructigena*, obtained from infected fruit, until run-off occurred. The fruit was transferred immediately to ripening rooms and examined after 5 days ripening at 68–70° F. On the six inoculated bunches, a total of twenty-two fingers were infected. Infection occurred at the stem-end, in a median position or at the blossom-end, and in some cases, symptoms were as advanced as those observed on naturally infected fruit (Pl. 3, fig. 1): diseased areas were invariably centred about old skin wounds. Uninoculated controls were not infected. The experiment demonstrated the rapidity with which infection by *T. fructigena* may progress, and also suggested that the fungus was acting predominantly as a wound parasite.

In another experiment, immature green Lacatan bananas were inoculated in various ways:

(*a*) Fingers with persistent stigmas were selected and each stigma was inoculated by making a small incision and applying conidia.

(*b*) Persistent stigmas were broken off and conidia applied to the fresh stigma scar.

(*c*) The surface of the skin at the blossom-end of the fruit was lightly scratched and inoculated.

(*d*) An inoculation similar to (*c*) was performed at a position about half-way along the finger.

(*e*) As in (*c*) and (*d*) but on the finger-stalk.

(*f*) Lesions infected with *Gloeosporium musarum* Cke. & Massee were inoculated by painting with conidia of *T. fructigena*.

(*g*) Apparently healthy, undamaged skin was inoculated by applying conidia with a soft brush.

The fruit was stored at 61–70° F. for 5–6 days, after which time it was fully ripe. The results of the inoculations are given in Table 1.

Table 1. *The infection of bananas by conidia of* Trachysphaera fructigena *after inoculating in different ways*

Type of inoculation	No. successful inoculations (out of 25)	Progress of infection
(*a*) Stigma wound-inoculated	20	Fair
(*b*) Stigma scar inoculated	25	Fair
(*c*) Fruit skin at blossom-end wound-inoculated	3	Very slight
(*d*) Skin half-way along finger wound-inoculated	15	Extensive
(*e*) Skin on finger-stalk wound-inoculated	11	Very slight
(*f*) *Gloeosporium musarum* lesions inoculated	0	—
(*g*) Healthy skin inoculated without wounding	0	—

An outstanding feature of these results was the rapid progress of infection after inoculation as in (*d*) (Pl. 3, fig. 2). Stigma and stigma-scar inoculations showed a higher percentage of infection, but did not develop so rapidly as the mid-finger inoculations. It is likely that skin tissues at the extreme blossom-end and in the finger-stalk offer greater resistance to infection than those at intermediate regions.

A further experiment was designed to test the ability of the fungus to infect apparently healthy, unwounded fruit maintained under laboratory conditions. Immature fingers were detached from the main-stalk and surface-sterilized by immersion for 2 min in 0·1 % mercuric chloride in 70 % alcohol. A glass ring, 1 cm. deep, was affixed about half-way along each finger, and, after introducing 1 ml. of a dense spore suspension of *T. fructigena*, it was sealed with a coverslip so as to form a modified Van Tieghem cell. By this arrangement, the spore suspension was maintained in contact with the skin of the fruit without drying-out occurring. The fruit was allowed to ripen for 14 days at 75° F., after which time there was no evidence of infection having occurred in any of the twenty replicates, even though a high percentage of spores had germinated in each cell.

In another experiment, one finger was detached from each of twenty

apparently healthy hands of immature Lacatan fruit. Each finger-stalk stump was inoculated immediately, by applying conidia of *T. fructigena* to the freshly exposed tissues. After 5–7 days ripening at 62–68° F., the fruit was full ripe. In no instance did the fungus invade the cushion. This result is compatible with the observation that the fungus does not normally pass from one finger to another via the cushion or main-stalk.

Discussion

Experimental inoculations carried out in ripening rooms have shown that *T. fructigena* readily infects immature Lacatan bananas when the skin tissue has been injured in some way. Symptoms of disease, which developed within a few days of inoculation, were often as advanced as those observed occasionally on Jamaican Lacatan fruit which had ripened in the United Kingdom. Since the fungus is not known to occur in the West Indies, and considering the fact that banana ships which carry Jamaican fruit do not normally visit West Africa, it seems reasonable to suggest that the natural infections were initiated after fruit had been discharged from the ships. Ripening rooms in which diseased Jamaican fruit was found were formerly occupied by severely infected Cameroon or Fernando Po fruit, and these would appear to be the most likely sources of inoculum.

Although the quantity of diseased Jamaican fruit observed so far is commercially insignificant, it is desirable that the risk of such infection should be reduced. It is normal practice to wash out ripening rooms before introducing a fresh consignment of fruit; nevertheless, it is now suggested that rooms formerly containing severely infected fruit should be disinfected more thoroughly. Similar considerations apply to chains and ropes by which stems are suspended. Also, all infected fruit should be removed as soon as it becomes evident, in order to minimize the amount of inoculum which may accumulate inside the room.

It was noted that naturally occurring infections of Jamaican fruit were usually associated with some form of skin injury. Also, experimental inoculation showed that the fungus was most active as a wound parasite and did not infect healthy, unwounded skin tissue. However, Brun & Merny (1947) observed infection of apparently healthy and uninjured Gros Michel bananas, 9–10 days after inoculation. An explanation of these conflicting results may be provided by further knowledge of varietal susceptibility and of the range of pathogenicity exhibited by different isolates of *T. fructigena*.

The author wishes to thank Messrs Elders & Fyffes Ltd., for kindly granting access to their banana ships and ripening stores, and for permission to use their fruit for experimental purposes; Prof. H. G. H. Kearns, O.B.E., for providing laboratory facilities at Long Ashton Research Station, University of Bristol; Mr D. E. Aliband, for photography.

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EXPLANATION OF PLATE 3

Fig. 1. Bananas infected with *Trachysphaera fructigena* 5 days after inoculation by spraying the entire bunch with a spore suspension.

Fig. 2. Effect of wound-inoculating immature bananas with *T. fructigena* at a point about halfway along the finger: symptoms developed after 5 days. Abundant conidial development.

(*Accepted for publication* 19 *May* 1959)

Fig. 1

Fig. 2

(*Facing p.* 104)

Trans. Brit. mycol. Soc. **43** (1), 105–110 (1960).

VISIBLE CHANGES IN THE VEGETATIVE MYCELIUM OF *COPRINUS LAGOPUS* FR. AT THE TIME OF FRUITING

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(With Plate 4 and 3 Text-figures)

Until 2 days old, diploid colonies of *Coprinus lagopus* in plate cultures are entirely composed of uniformly cylindrical, slender hyphae. After this, many hyphae immersed in the agar swell considerably, filling completely with dense contents which stain deeply with iodine-potassium iodide. When fruiting begins, vacuoles appear in the swollen cells, and enlarge until the latter are devoid of dense contents. The swollen cells are thus organs of storage in preparation for fruiting. Observations suggest that the exhaustion of their contents is brought about by protoplasmic streaming motivated by vacuolar pressure.

Similar storage cells develop in abundance in haploid colonies.

It has long been understood that fruiting in hymenomycetes is a process which must draw heavily upon materials contained in the vegetative mycelium (see, for example, Buller, 1931, 1933). Experimental proof of this in *Coprinus lagopus* was obtained by Madelin (1956*a*), who found that the quantity of material translocated from the vegetative mycelium to its developing fruit-bodies represents a large fraction of its total weight. For example, the dry weight of 12-day-old vegetative mycelium fell from 37·2 to 22 mg. in one day, while at the same time the developing crop of fruit-bodies increased by virtually the same amount. Because of the magnitude of this translocation it seemed possible that the hyphae of the vegetative mycelium might show visible change at this time. The results of an investigation into this possibility are presented below.

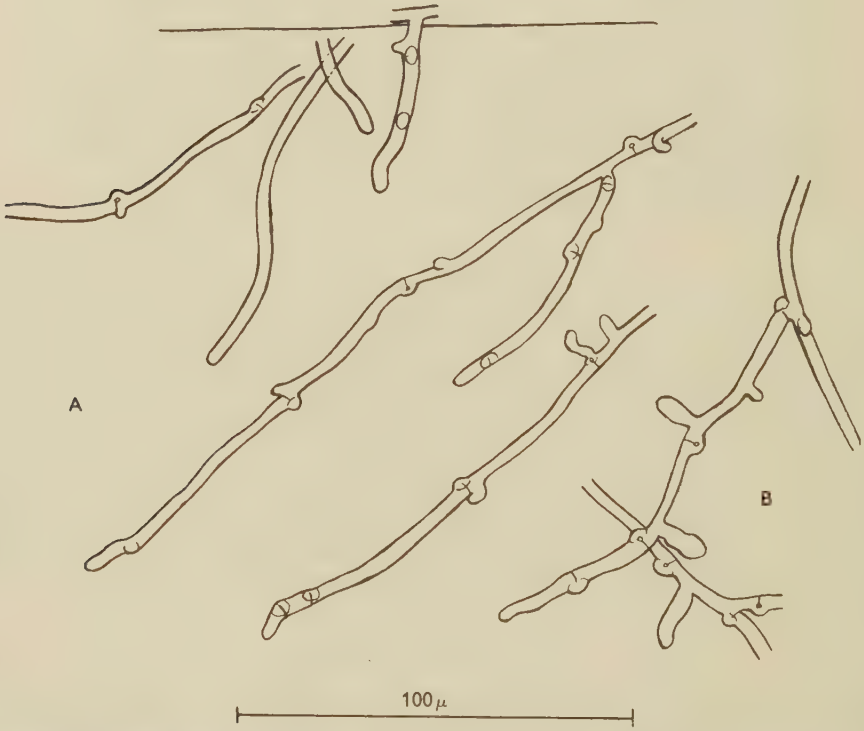
Material and methods

The diploid isolate of *C. lagopus* which was used was obtained by pairing complementary haplonts derived from the isolate used in earlier work (Madelin, 1956*a*, *b*). It grew and fruited well on the following synthetic medium: 10 g. glucose, 1 g. dl-α-alanine, 2 g. K_2HPO_4, 0·2 g. $MgSO_4.7H_2O$, 500 μg. thiamin hydrochloride, and 20 g. agar made up to 1 l. with distilled water.

Cultures were grown in absolutely flat-bottomed 9 cm. diam. Petri dishes, each containing 20 ml. of medium which provided a 3·1 mm. depth of agar. The plates were inoculated centrally with a small mycelial plug and incubated at 25° C. in continuous artificial light. Under these

conditions the fungus grew as a white, entirely vegetative colony for 7 or 8 days, by which time it was about 45 mm. in diam. On the 7th or 8th day the first few primordia of fruit-bodies became visible to the naked eye as minute white dots. Until the 11th day the primordia barely increased in size, but then they began to grow rapidly, fully maturing by the 15th or 16th day, by which time the colony had completely filled the plate.

A number of such cultures were prepared and at each of several different ages some were removed for microscopic examination. Radial strips of the agar were excised, and sections, approx. $\frac{1}{3}$ mm. thick, were cut by hand in various planes and mounted immediately in distilled water. After a first examination they were irrigated with IKI which stains glycogen yellow, through brown, to wine red according to its abundance, and proteins brown (Johansen, 1940). Such substances might be expected to be components of any material stored in the mycelium.



Text-fig. 1. Radial vertical section of agar plate culture 2 days old. A, Slender cylindrical hyphae near surface at margin of colony; B, hyphae from central region of colony. Note slightly swollen lateral branches. (All drawings done with camera lucida.)

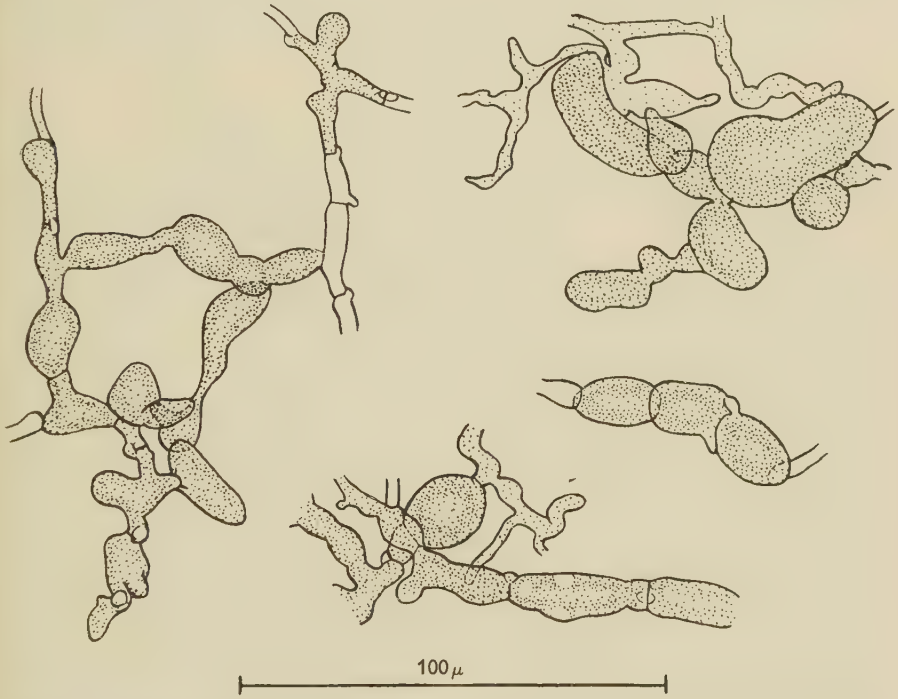
Results

The following is a summary of observations made on diploid cultures.

At 2 days old the colony was 5 mm. in diam., comprising a tuft of cottony aerial mycelium and a well-developed system of hyphae within the agar

(Text-fig. 4; Pl. 1, fig. 1). The whole colony, both above and within the substratum, was composed of more or less uniformly cylindrical hyphae. Within the agar, near the margin of the colony, these ranged from 1·8 to 3 μ wide. Nearer the centre more were of 3 μ width, and there also were present some short, slightly inflated, lateral branches up to 4·5 μ wide (Text-fig. 1 B).

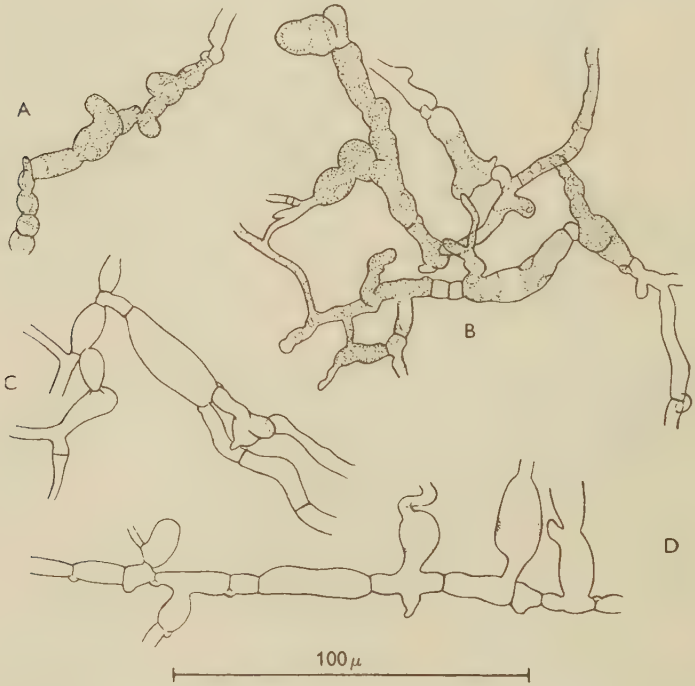
At 8 days old the 45 mm. diam. colony showed in its aerial mycelium and in the marginal region of the immersed part the same characteristics as the 2-day-old colony, namely, mycelium composed of more or less cylindrical hyphae. However, within the agar at distances greater than 5 mm. behind the growing margin were hyphae whose individual cells



Text-fig. 2. Radial vertical section of agar plate culture 7 days old. Four representative groups of immersed hyphae with variously swollen cells with dense, highly refractive contents.

were somewhat inflated, while beyond 7 mm. from the margin, a very large proportion of the cells were even more swollen and were completely filled with dense and highly refractive contents (Text-fig. 2; Pl. 4, figs. 2, 3). Most of these cells were 10–15 μ wide, while widths greater than 20 μ were not uncommon. Their contents stained golden-brown to deep reddish brown with IKI. Despite the swelling, clamp connexions were generally clearly visible. Not all of the immersed hyphae in the central region were altered in this way, for some, in whole or part, remained more or less cylindrical and slender. However, the immersed mycelium as a whole was characterized by the abundance of these variously inflated, sometimes

grossly distorted cells with their dense contents. Some represented modified parts of the original radially disposed hyphae which were the first to spread through the substratum (cf. Text-fig. 3D), but more were components of lateral branch systems which arose from these. Concerning the vertical distribution of these cells: none were seen in the aerial mycelium, the hyphae of which were uniformly slender, highly vacuolated and non-staining; many were present actually upon the surface of the agar; the great majority lay within 1·2 mm. of the surface; few lay deeper than 1·2 mm., and of these the contents were not conspicuously dense. It is probably owing to the absence of these swollen cells from young mycelia and from the aerial mycelium of mature colonies that illustrations of these



Text-fig. 3. Radial vertical sections of agar plate cultures. A and B, from colony in which fruiting has just started. Note the vacuolation of the swollen cells. C and D, from 17-day-old colony; swollen and normal cells now completely empty.

structures fail to appear in the works of earlier investigators of *Coprinus* such as Brefeld (1877) and Buller (1931, 1933) who figured essentially cylindrical hyphae.

During the period from the 11th to 16th days the sporophores were growing and maturing, and it was now that visible changes occurred within the swollen cells of the colony. As the sporophores grew, vacuoles appeared in the swollen cells and progressively enlarged, while the amount of deeply staining cytoplasm proportionally diminished, though the depth of staining of the residuum appeared to remain constant (Text-fig. 3A, B;

Pl. 4, figs. 4, 5). The process continued, until in 17-day-old cultures almost all of the formerly reserve-laden cells were now completely empty and barely visible and failed to stain (Text-fig. 3C, D; Pl. 4, fig. 6). During the period of their exhaustion the cells retained their globose or ellipsoidal form, but when wholly devoid of dense contents some lost their turgor and collapsed.

Observations on haploid colonies

A number of haploid cultures were examined in the same way, and it was found that here, too, cells of the immersed hyphae enlarged and became the seat of accumulation of dense and highly refractive reserve materials. The microscopic appearance of haploid colonies was, in respect of the appearance and abundance of swollen cells, essentially the same as that of diploid mycelia.

Discussion

The staining of the cytoplasm of the swollen cells suggests the presence therein of much glycogen and possibly also of protein. The exhaustion of these cells when fruit-bodies are being produced reveals their function as organs of storage in preparation for fruiting.

The observation that the stored materials are eliminated from these cells by displacement of the cytoplasm itself, accompanied by the expansion of vacuoles, instead of by a process of progressive dilution within the cytoplasm (for the depth of staining of the latter appears to remain constant), suggests that the mechanism of translocation is in fact protoplasmic streaming. Schütte (1956) found streaming to be associated with the ability to translocate in the fungi which he studied. The turgidity of the swollen storage cells of *C. lagopus* during the period of their exhaustion (Pl. 4, figs. 4–6) suggests that it is the pressure of the expanding vacuoles which plays a major role in the displacement of the food-laden cytoplasm. Buller (1933) suggested that vacuolar pressure is one of the causes of protoplasmic streaming in fungus mycelia, and also that it is responsible for the movement of nutriment towards the growing fruit-body in hymenomycetes (Buller, 1933, p. 149). The above observations on *C. lagopus* wholly support Buller's hypothesis. It is noteworthy that the aerial mycelium appears to play little part in the storage of materials for fruiting.

The presence of similar storage cells in haploid mycelia, which usually fruit tardily and abnormally, if at all (Buller, 1931), suggests that from the gross nutritive standpoint they are prepared for fruiting as early as are diploid colonies, but are prevented from doing so by other factors associated with their nuclear condition.

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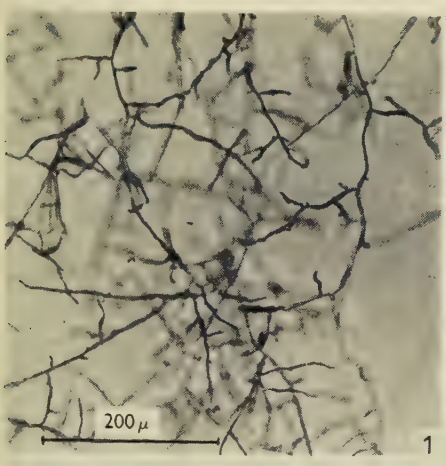
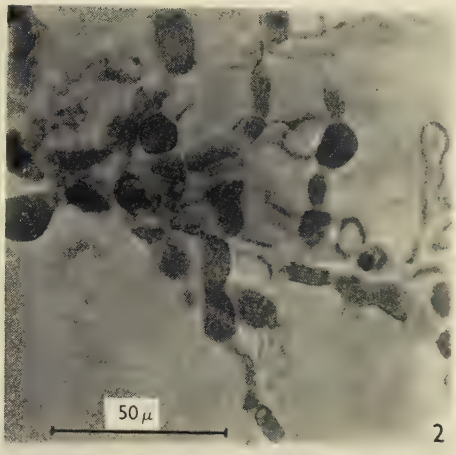
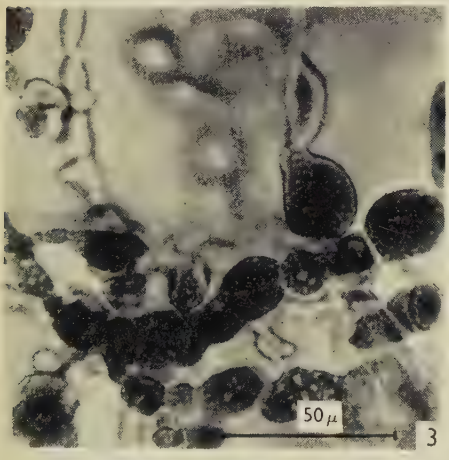
EXPLANATION OF PLATE 4

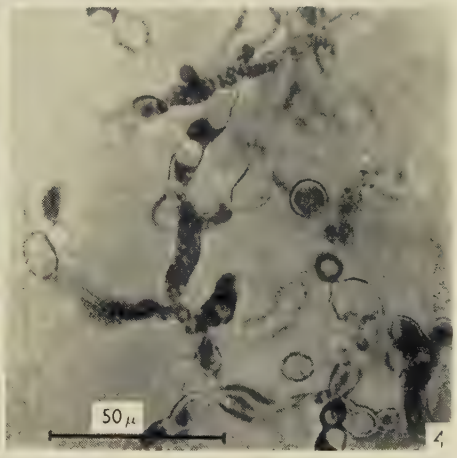
Photomicrographs of radial vertical sections of agar plate cultures, stained with iodine-potassium iodide.
Fig. 1. Immersed hyphae at centre of 2-day-old colony (low magnification).
Figs. 2, 3. 7-day-old culture; groups of swollen cells, deeply stained with iodine.
Figs. 4, 5. Colony in which fruiting has just commenced; swollen cells becoming increasingly vacuolated.
Fig. 6. 17-day-old colony; cells now completely empty.

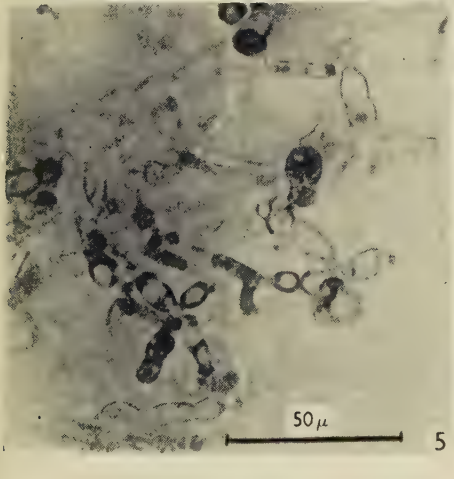
(*Accepted for publication* 9 *May* 1959)

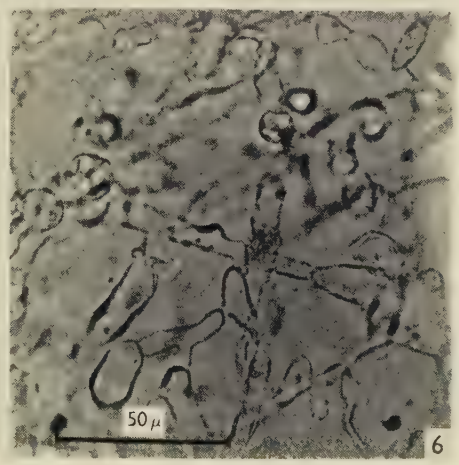












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Trans. Brit. mycol. Soc. **43** (1), 111–116 (1960).

EFFECT OF CARBON DISULPHIDE FUMIGATION ON *TRICHODERMA VIRIDE* AND OTHER SOIL FUNGI

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Botany School, University of Cambridge

The effect of carbon disulphide fumigation on the soil fungus flora has been studied over the range 0·05–20·0 ml. carbon disulphide/250 g. soil in Evans soil recolonization tubes. Soil samples were plated out at intervals after fumigation, and the commoner fungi developing on the soil plates were recorded. *Trichoderma viride* became the dominant fungus after fumigation when the dosage range of carbon disulphide was 0·05–0·1 ml./250 g. soil. The tolerance of *T. viride* to carbon disulphide was lower than that of *Penicillium nigricans* and *P. citrinum*, and much lower than that of the most resistant fungi, which were *Aspergillus fumigatus*, *A. fischeri*, *Penicillium luteum* and several unidentified species. The frequency with which *Trichoderma viride* has been reported as a recolonizer of fumigated soils is attributed as much to its high growth rate as to its tolerance of carbon disulphide.

The investigation described in this paper was undertaken in continuation of that carried out by Evans (1955) in this laboratory. In an experiment with carbon disulphide as a soil fumigant, Evans found that *Trichoderma viride* subsequently became dominant in the treated soil when the initial concentration of carbon disulphide did not exceed 0·5 ml./250 g. soil. After higher doses of carbon disulphide, the dominant fungal recolonizers were two ascosporic fungi, *Penicillium luteum* and *Aspergillus fischeri*. Evans was able to correlate these findings with the degree of tolerance shown by the various species to the action of carbon disulphide *in vitro*.

Particular interest in the efflorescence of *Trichoderma viride* in soils previously fumigated with a suitable dosage of carbon disulphide or other fumigant was aroused by the hypothesis of Bliss (1951), which postulated that the killing of *Armillaria mellea* in infected citrus roots by carbon disulphide fumigation was due not to direct fungicidal action but to the subsequent lethal activity of *Trichoderma viride*. The experiments of Garrett (1957, 1958) suggested that Bliss's hypothesis was partially correct, inasmuch as direct fungicidal action of carbon disulphide on *A. mellea* was followed and extended by the lethal activity of *T. viride*. These findings call for further work on this microbiological effect, to determine more precisely the range of fumigant dosage that promotes subsequent dominance of *T. viride*, and to study the sequence of events that results in dominance.

Experimental work

The soil employed in all experiments was Kettering loam, which had been used both by Evans (1955) and by Garrett (1957, 1958) in earlier phases of this study. Experiments were carried out in the 'soil recolonization tube'

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devised by Evans (1955). This apparatus consists of an 18 in. length of 'Pyrex' glass tubing, of 1 in. internal diameter, to which seven side-arms, 1 in. long and 0·5 in. internal diameter, are fused at 2 in. intervals. Each recolonization tube received 250 g. air-dry Kettering soil, previously sieved (4 mm. mesh), in ten equal instalments of 25 g. After each addition of soil, the correct volume of distilled water to bring the soil moisture content to 37·5 % M.H.C. was pipetted into the tube. On completion of filling, all openings were tightly closed by rubber bungs, and the tube was left for 48 hr. to allow the soil moisture content to equilibrate. The required dose of carbon disulphide was then pipetted into each tube through its centre side-arm, after which all rubber bungs were tightly secured in the side-arms and ends of the recolonization tube by binding with Sellotape. Fumigation of the soil was maintained for 96 hr. in a thermostat at 22·5° C. The rubber bungs were then removed and replaced by 'Oxoid' metal caps, which permitted diffusion of the fumigant out of the soil without exposure of the tube to microbial contamination. Thereafter, tubes continued to be incubated at 22·5° C.

At intervals, soil samples were taken with a sterile nichrome inoculating needle from each of the seven side-arms of each recolonization tube, and plated out on soil plates (Warcup, 1950). A suitable dosage range of carbon disulphide was covered by means of three consecutive experiments, for which sampling periods (calculated from the end of the fumigation period of 96 hr.) were as follows: 0·05–0·2 ml. carbon disulphide, 10 and 22 days; 0·5–1·5 ml., 9, 21, 32 and 42 days; 2·5–20·0 ml., 10, 18, 32 and 40 days. A complete record of all the fungi identified on the soil plates made from these soil samples would have been too bulky to present, but a record of all the commoner species is given in Table 1. For each species is given, at each dosage of carbon disulphide, the total number of side-arms (out of a possible maximum of 7) from which it was isolated, at one or more of the sampling dates.

Before discussing the results set out in Table 1, it is necessary to give a short list of the commoner soil fungi identified on soil plates made from untreated Kettering soil. These included *Penicillium citrinum*, *P. nigricans*, *Mucor* spp., *Zygorhynchus vuillemini*, *Absidia spinosa*, *Mortierella sepedonioides*, *Gliocladium roseum*, *Fusarium oxysporum*, *F. culmorum*, *Chaetomium globosum* and *Stysanus medius*. *Trichoderma viride* was also common, being seen on 30–40 % of soil plates.

The fungal species listed in Table 1 have been arranged in order of tolerance to the action of carbon disulphide. From this list, it appears that the tolerance of *Trichoderma viride* to carbon disulphide is only moderate, by comparison with the high degree of tolerance shown by *Aspergillus fumigatus*, *A. fischeri*, and *Penicillium luteum*. Two chlamydospore-forming species, which have not been identified (nos. 36 and 39), were also highly tolerant of carbon disulphide, as were some unidentified species of *Penicillium*.

In these experiments, the dosage range of carbon disulphide giving maximum abundance of *Trichoderma viride* developing in the fumigated soil was 0·05–0·1 ml./250 g. soil. This does not exclude the possibility that, after field fumigation, *T. viride* may invade a volume of soil receiving

a higher dose than this, from inocula sufficiently remote from the injection points to survive fumigation. It therefore seemed desirable to test the speed with which *T. viride* can colonize a volume of sterilized soil, free from fumigant, in competition with other fungi, and so the following experiment was set up.

Table 1. *Number of side-arms (out of a possible maximum of 7) of Evans soil recolonization tube from which various fungal species were recorded, after injection of carbon disulphide into the centre side-arm*

	ml. CS_2/250 g. soil									
	0·05	0·1	0·2	0·5	1·0	1·5	2·5	5·0	10·0	20·0
Mucor spinosus	4	0	0	0	0	0	0	0	0	0
Fusarium culmorum	4	1	0	0	0	0	0	0	0	0
Absidia spinosa	4	2	3	0	0	0	0	0	0	0
Zygorhynchus vuillemini	5	2	2	1	1	0	0	0	0	0
Fusarium oxysporum	4	4	2	2	0	0	0	0	0	0
Trichoderma viride	5	5	4	1	0	0	0	0	0	0
Mortierella sepedonioides	0	0	0	2	2	0	0	0	0	0
Penicillium nigricans	2	5	2	3	3	0	0	0	0	0
Stysanus medius	0	0	0	1	1	0	0	0	0	0
Penicillium citrinum	7	6	7	7	7	3	3	2	0	0
Aspergillus fumigatus	1	0	0	0	1	1	6	2	1	7
A. fischeri	0	0	0	0	0	1	2	1	2	0
Penicillium luteum	0	0	0	0	0	1	2	0	1	0
No. 39	0	0	0	0	0	0	2	4	0	2
No. 36	0	0	0	0	0	0	0	2	0	2

Table 2. *The spread of seven fungi from their original inoculation points in the seven side-arms of an Evans soil recolonization tube filled with steam-sterilized soil*

Side-arm no.	Fungus originally inoculated	Fungi isolated			
		After 6 days	After 12 days	After 25 days	After 37 days
1	*P. nigricans**	*P. nigricans*	*P. nigricans*	*P. nigricans*, *T. viride*	*T. viride*, *P. nigricans*
2	*A. fischeri*	*A. fischeri*	*A. fischeri*, *T. viride*, *M. spinosus*	*T. viride*, *A. fischeri*, *M. spinosus*	*T. viride*, *M. spinosus*, *A. fischeri*
3	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	*F. oxysporum*	*T. viride*, *F. oxysporum*
4	*T. viride*	*T. viride*	*T. viride*	*T. viride*	*T. viride*, *F. oxysporum*
5	*P. citrinum*	*P. citrinum*	*P. citrinum*, *T. viride*	*P. citrinum*, *T. viride*	*T. viride*, *P. citrinum*
6	*F. culmorum*	*F. culmorum*	*F. culmorum*, *P. citrinum*	*F. culmorum*, *P. citrinum*	*T. viride*, *P. citrinum*, *F. culmorum*
7	*M. spinosus*	*M. spinosus*	*M. spinosus*, *P. citrinum*, *T. viride*	*T. viride*, *P. citrinum*, *M. spinosus*	*T. viride*, *P. citrinum*, *M. spinosus*

* For full binomials of these seven species, see Table 1.

A soil recolonization tube was filled with Kettering soil at 37·5 % M.H.C., as described above, and steamed for 1 hr. on 3 successive days. Seven

fungi were then inoculated, each one individually into one of the seven side-arms, using an agar disk carrying sporulating mycelium. Side-arms and ends of the tubes were covered with 'Oxoid' caps. The recolonization tube was incubated at 22·5° C., and samples of soil from each side-arm plated out at intervals, with results shown in Table 2; the various species recorded from each side-arm are listed in order of their abundance at each sampling on the soil plates. Comment on these results is reserved for the Discussion.

Discussion

In Table 3 are set out for comparison the results obtained in four different investigations, including the present one, on the effect of carbon disulphide fumigation upon the subsequent development of *Trichoderma viride* in the fumigated soil.

Table 3. *Optimum concentration of carbon disulphide for subsequent dominance of* Trichoderma viride *in fumigated soil*

Author	ml. CS_2/ 1000 ml. container space	Dosage time (hr.)	Method used for estimating subsequent dominance of *T. viride*
Evans (1955)	2.28	24	Plating soil samples on Warcup soil plates
Bliss (1951)	0·23	168	Plating out root samples
Garrett (1957)	0·42	96	Observation of green plaques of sporulating *T. viride* at soil-glass interface
Present investigation	0·23–0·46	96	Plating soil samples on Warcup soil plates

Table 3 shows that the results of the present investigation agree closely with those obtained by Bliss (1951) and by Garrett (1957) in suggesting that, for a dosage time of 96 hr. or more, a concentration of carbon disulphide corresponding to 0·25–0·5 ml./1000 ml. container space is near the optimum for promoting subsequent dominance of *T. viride* in the recolonized soil. Evans (1955) obtained dominance of *T. viride* on his soil plates after a considerably higher dose of carbon disulphide—2·28 ml./1000 ml. container space—but he employed a much shorter dosage time, of only 24 hr.; this concentration of carbon disulphide was the lowest actually tested by Evans.

In the light of these findings concerning the degree of tolerance shown by *T. viride* to carbon disulphide in comparison with that of other soil fungi some revision of our ideas concerning the ecology of this fungus in fumigated soils becomes inevitable. Garrett (1956, p. 16) has summarized the earlier evidence, which indicated that *T. viride* commonly became dominant after soil treatment with a variety of fumigants and fungitoxic compounds. At that time, it was still possible to attribute this dominance of *T. viride* simply to its tolerance of fungicidal and fungistatic compounds. The present investigation has now shown, however, that the degree of tolerance towards carbon disulphide shown by *T. viride* is by no means exceptional amongst soil fungi, and that this factor alone is quite insufficient to explain its frequently reported dominance in fumigated soils.

The probability that *T. viride* (or any other fungus) will become dominant in a fumigated soil will be largely determined by three characteristics of the fungus:

(1) Its degree of tolerance to the fungicidal action of the fumigant. This will determine the proportion of fungal propagules that survives soil fumigation, and will be able to initiate new mycelial growth after the fumigant has dispersed.

(2) Its degree of tolerance to fungistatic effects of the fumigant. This will determine the earliest time at which fungal propagules surviving fumigation can initiate new growth as the concentration of fumigant falls during dispersal, after fumigation has been completed.

(3) Its intrinsic growth rate. This will determine the radius of the fungal colony developing from each surviving propagule, in the zone around it that has been sterilized by fumigant action, before its further expansion is arrested by the development of surrounding colonies.

The foregoing analysis of the situation in a soil immediately after fumigation suggests that *T. viride* owes its success in recolonization to the conjunction of a moderate degree of fumigant tolerance and an intrinsically high growth rate. Some fungi, e.g. many phycomycetes, grow as rapidly as *T. viride* but are significantly less tolerant of fumigants. Other fungi, e.g. *Penicillium* spp. of the *luteum* series and *Aspergillus fischeri*, are much more tolerant of fumigants than is *T. viride* but are much slower growers. In the race for recolonization of fumigated soil, the resistant Penicillia and Aspergilli provide many early starters, but their longer start in this race is insufficient and their young colonies are quickly overtaken by the colonies of *T. viride* starting later. Many fast-growing phycomycetes, on the other hand, are so severely handicapped by their low tolerance of the fumigant that they are either eliminated before the start of the race (fungicidal effect), or else are delayed too long at the starting line (fungistatic effect).

This interpretation of the situation developing in fumigated soils is illustrated in part by the experimental results given in Table 2. In a soil recolonization tube sterilized by intermittent steaming, *T. viride* was inoculated into the centre side-arm and six other species individually into one each of the other six side-arms. *T. viride* was found in three other side-arms besides its own after 12 days, in the fourth side-arm after 25 days, and in the fifth and sixth side-arms after 37 days. None of the other six fungi showed so wide a spread from its original side-arm; *P. citrinum* established itself in two other side-arms besides its own, and *Mucor spinosus* and *Fusarium oxysporum* each succeeded in colonizing one other side-arm. Successful colonization of other side-arms in this experiment must have necessitated rapid growth by a fungus out of its own side-arm, along the main body of the recolonization tube in either direction, and up other side-arms before the original inoculant had completely occupied the habitat. It is possible that conditions in freshly steamed soil, with a mounting concentration of ammonium salts and other solutes, are more congenial to *T. viride* than to some of its competitors, and that this may help to explain the frequent dominance of this fungus in recently steamed soils (Garrett, 1956, p. 17). Warcup (1951) has shown that the heat tolerance of *T. viride* is not outstanding, and so dominance of this fungus in

steamed soils cannot be attributed to a survival of the actual steaming treatment that is proportionately higher than that of its competitors.

The experiments described in this paper thus permit the conclusion that the dominance of *T. viride* in soil fumigated with carbon disulphide can be attributed to a moderate degree of tolerance to the fumigant allied with an intrinsically high growth rate. This enables the fungus to colonize volumes of soil sterilized by the fumigant in advance of most of its fungal competitors. These two factors may not be the only ones concerned in the success of *T. viride* in recolonization; the results given in Table 2 indeed suggest that *T. viride* may infiltrate the colonies of other fungi when growing in soil as frequently as it can be observed to do when growing on agar plates in the laboratory.

The author wishes to acknowledge his indebtedness to Dr S. D. Garrett for kindly suggesting this line of work and for his helpful advice and interest during the course of this investigation. Grateful acknowledgements are also made to Prof. G. E. Briggs, F.R.S., for providing facilities, and to the British Council for a travel grant. The writer also thanks Prof. N. F. Robertson for helping him with identification of some of the fungi.

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(*Accepted for publication* 1 *June* 1959)

Trans. Brit. mycol. Soc. **43** (1), 117–125 (1960).

THE INFECTION OF BARLEY BY LOOSE SMUT (*USTILAGO NUDA* (JENS.) ROSTR.)

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(With Plates 5 and 6 and 1 Text-figure)

The entry of *Ustilago nuda* into the barley ovary and the subsequent passage of the fungus to the embryo have been investigated in inoculated and naturally infected seed. The results were the same for both. The hyphae penetrate the ovary wall (pericarp), traverse the parenchyma, pass along the integuments (testa) on the ventral side of the grain and cross the endosperm at the base of the grain and so enter the scutellum. From the scutellum the mycelium permeates most parts of the embryo, being most plentiful in the hypocotyl and growing point region. Infection via the style has not been found.

The infection of wheat with *U. tritici* is compared. Mycelium is mainly intracellular in the pericarp and testa, and mainly intercellular in the aleurone, endosperm, scutellum and embryo.

Lang (1917) gave the first detailed account of the entry of *Ustilago nuda* (Jens.) Rostr. into the ovary and also of its subsequent path within the developing grain. He suggested two ways in which the fungus may reach the embryo; either the spores germinate on the style and the hyphae follow the path of the pollen tubes to the integuments, which they then follow to the embryo, or the spores germinate on the surface of the ovary wall and the hyphae grow between the integuments and nucellar epidermis to the embryo. Other relevant literature has previously been summarized (Batts, 1955). Since this work was started, Pedersen (1956) found that the hyphae always penetrate the pericarp and then enter the integuments, which they follow to the scutellum and embryo. He considered that 'the previously held theory that barley may be infected with *U. nuda* through the styles should be discarded'.

Materials and methods

In the summer of 1955, a number of ears of barley varieties susceptible to loose smut were artificially inoculated by the partial vacuum method (Moore, 1936; Oort, 1939). The ears were inoculated at anthesis and collected at intervals of 6, 11, 18, 26 and 33 days. The material was fixed in formalin-acetic-alcohol and subsequently preserved in 50% alcohol.

In 1957, the varieties Plumage Archer, Proctor and Carlsberg were inoculated at anthesis by the hypodermic needle method (Poehlman, 1945). The inoculum was obtained from Fresia, a spring barley, and

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comprised 0·5 g. spores in 100 ml. water. Inoculated ears of the three varieties were collected after 24 and 48 hr., and then after 4, 6, 11, 26 and 33 days. The ears were fixed and preserved as detailed above.

To obtain naturally infected material, ears adjacent to smutted plants of Carlsberg and Fresia were collected from severely affected plots in the summer of 1957, fixed and preserved as above.

Grains were examined by three methods:

(i) *Surface preparation.* The various parts of the grain, i.e. pericarp, testa, aleurone and embryo, were separated and examined by mounting the layers stained in cotton blue in lactophenol. About 350 grains were examined in this way.

(ii) *Microtome sections.* Transverse and longitudinal serial sections (12 μ thick) were made of the inoculated grains collected at different intervals. Most sections were stained with Carbol thionine blue counterstained by Orange G. (Johansen, 1940), or with Pianeze III b (Vaughan, 1914) and Carbol thionin (Stoughton, 1930), counterstained with Orange G. About 100 grains were examined in this way.

(iii) *Freezing microtome sections.* Transverse and longitudinal sections (15 μ thick) were made and mounted in cotton blue in lactophenol. About 100 grains were examined in this way.

The terminology of Hector (1936) has been used in describing grain development.

Observations on artificially inoculated grains

Observations on the Carlsberg material inoculated in 1955 and 1957 agreed in all respects and the results are treated together. The observations made on the path of infection in Plumage Archer and Proctor agreed with those made on Carlsberg, except for a few minor differences which are indicated.

After 24 hr. the ovary had begun to swell at the top, and it was then approximately 2·0 mm. long × 1·5 mm. broad × 1·0 mm. thick. It was still pale green in colour. The branches of the stigma were covered with pollen grains and loose smut spores, and were fresh and turgid, showing no sign of desiccation. Hairs were present over the ovary, being most plentiful on the shoulders. The greatest number of spores was found on the shoulders. There was very little cell differentiation at this stage. The cells were parenchymatous and stained deeply with cotton blue and were filled with protoplasm.

More germinating spores were present on the ovary wall than on the style and a count gave the following results:

percentage of spores germinating on the ovary wall, 65;
percentage of spores germinating on the style, 25.

A few spores were enveloped by long promycelia. Only a few penetrations were observed. On coming in contact with the epidermal cells of the ovary wall, the tips of the promycelia developed appressorium-like swellings, through which the hyphae penetrated the cells immediately below (Pl. 5, fig. 1).

After 48 hr. there was little further development, but many penetrations on the ovary were observed, and hyphae were seen in the subepidermal layer of the ovary (Pl. 6, fig. 6).

After 6 days the ovary had elongated considerably and it was then approximately 7·0 × 2·0 × 1·5 mm. The stigmas were still fresh and turgid. The serial sections clearly revealed the positions of the integuments, which were found below several layers of thin elongated cells constituting the subepidermis and epidermis of the pericarp or the seed coat of the grain. The integuments were surrounding the developing embryo and the endosperm, which at this stage occupied a very small portion of the developing grain. Cell differentiation was still not obvious, all the cells being thin walled, full of protoplasm and deeply stained by cotton blue.

At this stage, long promycelia were present on the surface of the ovary wall and many penetration points were present all over the ovary wall but especially on the shoulders. A few promycelia were still connected with their spores. The spores retained their brown coloration and it was not possible to differentiate between germinated and ungerminated spores.

In one or two grains the hyphae had reached the integuments, but most of the mycelium was in the layer of cells which constituted the pericarp. Many of the hyphae ran parallel to one another towards the integuments, from closely situated foci of infection. The penetrating hyphae produced no obvious malformation or distortion of the cell wall or of the structure of the cell.

After 11 days, considerable development of the ovary had taken place and it had reached its maximum length, measuring approximately 9·0 × 2·5 × 2·0 mm. The endosperm, surrounded by the developing testa derived from the integuments, was a narrow elongated area occupying more than half the grain. The cells of the outer layer of the inner integuments were still discernible, but the cells of the outer integument had disappeared. Cuticle was developing on the outer layer of the inner integument and pigment was being deposited in the cells of the inner layer of the inner integument (the future coloured layer of the testa). The cells of the nucellus were, in the main, discernible. The aleurone cells surrounding the endosperm were two to three layers thick, elongated and thin walled. The cells of the pericarp contained starch grains. The embryo was lenticular in shape, and the coleoptile showed as a small projection.

Mycelium was present on the outside of the pericarp and there were numerous penetration points all over the ovary wall (Pl. 5, fig. 1). In about 80% of the seeds examined, mycelium was present over most of the pericarp, its greatest density being on the shoulders, just beneath the lowest of the hairs, and along the crease. No mycelium was seen in the area of the style. The fungus was usually found to have crossed the pericarp in a centripetal direction and entered the developing testa and nucellus. On reaching the integumental layer the hyphae branched off and proceeded through this towards the embryo.

After 18 days, the size of the grain was approximately 9·0 × 3·0 × 2·5 mm. Increase in width was most rapid at this time, and proceeded until about the 20th day. The expansion of the endosperm had continued and it occupied more than three-quarters of the grain. The cells of the outer

layer of the inner integument had disappeared as well as those of the outer integument. The developing testa was pigmented mostly in the inner layer of the inner integument. The cells of the outer layer were compressed. The nucellus cells were not discernible in some of the sections examined. The aleurone layer surrounding the endosperm was 3–4 cells wide, each cell being almost square in cross-section and thin-walled. A layer of collapsed cells was present between the endosperm and the outer cell layer of the scutellum. This layer consisted of long epithelial cells devoid of starch. The pericarp parenchyma was considerably reduced and cell thickening was in progress. The future cross-cells still retained their contents. In the embryo, the plumule and root initials were visible. By this time the lemma and palea had fused with the seed coat of the grain.

More penetration points were present on the outside of the pericarp, and the hyphae usually grew in a direct route from the surface of the pericarp to the integuments and produced very few branches. The hyphae seemed to have no difficulty in penetrating the subsequent cells. Having reached the testa, the mycelium grew towards the embryo. The fungus was present to some extent in the young aleurone, in the endosperm towards the base of the grain, and in the collapsed cells lying between the endosperm and the outer cell layer of the scutellum (Pl. 5, fig. 4). In a few grains, mycelium had passed to the epithelial layer of the scutellum. More mycelium was present in the ventral side than in the dorsal side of the grain.

After 26 days, the size of the grain was approximately $9{\cdot}5 \times 3{\cdot}5 \times 2{\cdot}5$ mm. and the development was in general only a little behind that at 33 days. Mycelium was plentiful on the outside of the pericarp, which showed many penetration points. Parts of the testa were heavily infected with mycelium. In about 60 % of the grains examined, mycelium had reached the scutellum, and in a few grains mycelium had even reached the base of the coleoptile and coleorhiza.

After 33 days, considerable development of the grain had taken place; it was approaching its mature form, and measured approximately $9{\cdot}8 \times 3{\cdot}5 \times 2{\cdot}5$ mm. Except for the embryo, the whole grain was occupied by starch-filled endosperm cells. The pericarp was smooth on the dorsal side and folded inwards, making a furrow on the ventral side of the grain. It was made up of an outer and an inner epidermis with 3–4 layers of cells between. The outer epidermis (epicarp) consisted of elongated cells with ends usually pointed. The inner epidermis consisted of 4–5 layers of parenchymatous cells similar to those of the epicarp. The pericarp appeared beaded in surface view. The testa, which imparts colour to the seed, was derived from the inner of the two integuments, and consisted of two layers of elongated cells which were crushed on the dorsal side of the grain, and folded into a crease on the ventral side where it joined the funicle of the seed. Except in the crease region, the cells were compressed into a very narrow band on the inside of the pericarp, the inner layer of which was pigmented where the cells were 3–4 layers thick. The aleurone layer, surrounding the endosperm and closely following the contours of the seed coat, was 3–4 cells thick, except on the dorsal side near the scutellum, where it was one cell thick. Over the plumule and coleorhiza, it was very

thin and shrivelled and was difficult to recognize as a distinct layer. The individual cells were nearly square in cross-section. The nucellar layer was a narrow, structureless mass between the testa and the aleurone.

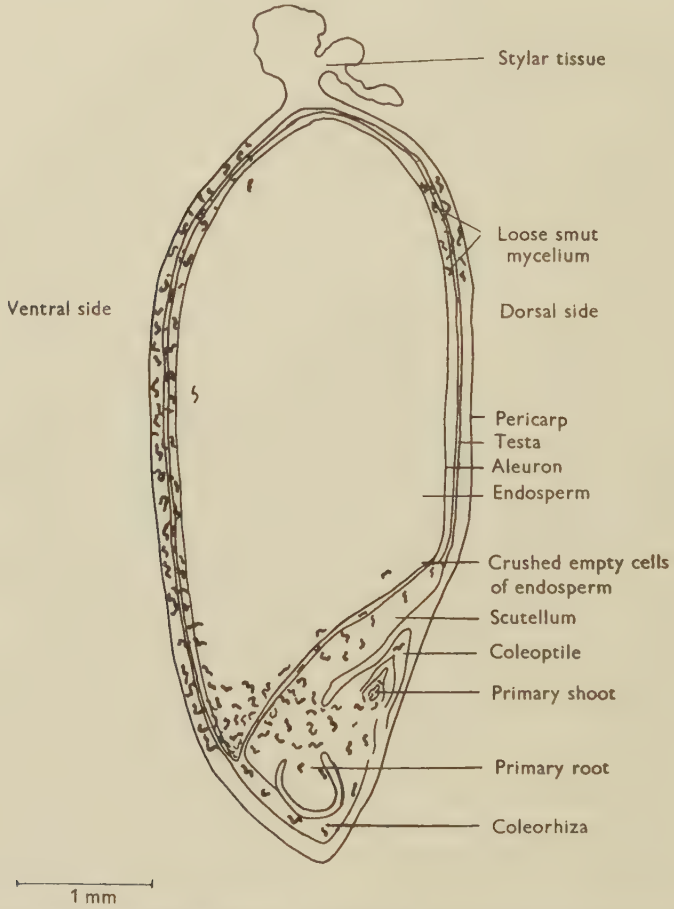
The embryo, on the dorsal side at the base of the grain, was in close contact with the endosperm, and on the other was covered by the pericarp and testa. It was a highly differentiated structure possessing a short hypocotyl, at the apex of which was the plumule or primary bud, which consisted of rudimentary leaves surrounded by the coleoptile. The root system enclosed by the coleorhiza was at the base of the embryo. On the side adjacent to the endosperm was the scutellum, which was slightly curved, the convex surface fitting closely to the endosperm. The epidermis of the scutellum, in contact with the endosperm, consisted of elongated cylindrical cells known as the epithelial layer, the long axes of which were arranged at right angles to the curved surface of the scutellum. The cells of the endosperm parenchyma, in contact with the epithelial layer of the scutellum, were crushed together.

At this stage, the whole path of development of the fungus can be followed (Text-fig. 1). Penetration points were bold and easily found on the pericarp. Large areas of the pericarp were invaded by the mycelium, particularly along the crease region. Certain areas of the testa, especially on the crease region, were completely permeated with mycelium. Numerous peg-like processes invaginated the contents of the testa. Mycelium in the testa region grew towards the base of the grain. At this stage, mycelium was frequently found in the aleurone layer (Pl. 5, fig. 3). Pieces of mycelium were frequently present in the endosperm towards the periphery, but were never seen in the middle of the endosperm. In most of the grains examined, mycelium was present at the base of the endosperm where it joined the crease (Pl. 5, fig. 4), very little being found on the dorsal side. On the ventral side the fungus passed from the testa, over the aleurone and endosperm to the scutellum (Pl. 5, fig. 4). Mycelium was present in the layer of collapsed cells lying between the endosperm and the outer cell layer of the scutellum. In the scutellum the mycelium had formed a network, and in some grains large areas were completely interwoven with hyphae. The mycelium then proceeded further and permeated parts of the embryo, being most plentiful in the hypocotyl and the growing point region (Pl. 6, fig. 5). Hyphae were also observed in the leaf primordia and a few were also found in the root primordia. The host cells seemed to accommodate the invading hyphae without showing any adverse effects, and no disarrangement or malformation of the cells of the scutellum and embryo were seen.

The stigmas were removed from some florets at anthesis, and measured under the microscope. They ranged in length from 2500 to 3000 μ. Thus the germ tube of a spore, germinating towards the tip of a stigma branch, would have to traverse this very considerable distance before reaching the tissues of the barley ovary; entry from the ovary wall is a matter of only a few microns.

The observations made on the path of infection in Plumage Archer and Proctor varieties were in agreement with those made on Carlsberg, except for a few minor differences. In Plumage Archer and Proctor, browning

and death of epidermal cells were often associated with infection, three or four cells around the point of infection being brown and distorted. Thickening of cell walls in the regions of penetration was also noted. The path of infection and the position of the mycelium in the embryo were, however, identical with those in Carlsberg.



Text-fig. 1. Diagrammatic representation (longitudinal section) of the position of mycelium in an infected barley grain, 33 days after inoculation.

Observations on naturally infected grains

About fifty mature grains from a plot of infected Fresia barley were examined by surface preparations and by serial sections. Six were found to be infected. The grains averaged 9·0 × 4·0 × 3·0 mm.; the stage of growth was approximately 6 weeks after anthesis, and the structure was similar to that described above for 33 days.

Spores and numerous pollen grains were present on the stigma, the brushes and on the upper half of the ovary wall. The remains of the style

and stigma were still present, but no mycelium was present on them. Spores, mycelium and penetration points were plentiful on the pericarp. Mycelium was also plentiful in the testa, with scattered pieces in the aleurone. Lengths of hyphae were found in the endosperm towards the periphery, but not in the middle of the endosperm. In the embryo, mycelium was present in the scutellum and in the growing point region. On the ventral side of the grain, the fungus passed from the testa over the aleurone and endosperm to the scutellum, and penetrated the embryo proper. Very little mycelium was present on the dorsal side of the grain.

In sections of one grain, mycelium was present in the tissue of the base of the style, but this mycelium did not penetrate the grain any further. Penetration points were present on the shoulders of this infected grain.

The distribution of mycelium in the naturally infected grain was therefore identical with that found in artificially inoculated grain, and there was no indication that the path of infection had been different in any way.

Comparison of infection in wheat and barley

In general, the infection of barley by loose smut was very similar to that of wheat, but there were some interesting differences. The penetrating hyphae were much finer in barley than in wheat. Batts (1955) found mycelium on the dorsal side of the grain, and observed that the fungus grew towards the base of the grain and entered the embryo. In barley, mycelium was always observed on the ventral side of the grain and invariably entered the scutellum and embryo via the layer of endosperm at the base of the grain. In wheat, hyphae rarely penetrated the endosperm, but in barley mycelium was frequently observed in the outer layer of the endosperm, especially on the ventral side. In wheat, mycelium was frequently seen on the ventral side of the grain, but it did not lead to infection of the scutellum and embryo. In barley, mycelium was sometimes found on the upper half of the dorsal side of the grain, but it did not lead to infection of the embryo.

Discussion on path of infection

The results obtained are in close agreement with those of Pedersen (1956), who has discussed his results in relation to previous observations.

It now appears doubtful whether any fungus pathogen reaches an ovary via the stigma and style. Campbell (1958) disproves previous conclusions that *Claviceps purpurea* enters cereal grain via the style, and Jung (1956) inoculated the stigmas of sixty-one plant species with numerous fungi, but never found entry to the ovary effected by this route. He found that the style tissue had an inhibitory action on fungal development.

Host–parasite relationships

Penetration was through any part of the epidermis of the ovary wall, but was most frequent on the shoulders and crease. It was most often through the centre of the cell wall but was sometimes between the vertical walls

of the epidermal cells. Some swelling of the adjacent cell walls usually occurred. At the point of entry an appressorium was formed, beneath which a bulbous swelling developed, and through this the hyphae passed in a constricted condition into the cell immediately below; a funnel-like structure resulted (Pl. 6, fig. 7). In the formation of these funnels, the reaction of the host appeared to be such that the cuticle and cell wall were invaginated. The structure of the funnel was not homogeneous; approximately in the centre of the funnel was a somewhat darker area, which as far as can be judged was the space between the cuticle and cell wall. Near the surface of the funnel, the cuticle was separated further from the cell wall. The presence of the fungus appeared to activate the host in such a way that the funnel structure developed as a continuation of the invaginated cuticle and cell wall. The mycelium assumed its normal diameter after passing through the funnel. Sheathing of the internal mycelium was extremely common (Pl. 5, fig. 2; Pl. 6, fig. 6).

Mycelium was mainly intracellular in the pericarp and testa. An appressorium was formed on each wall at the point of penetration, sheathing often being present around the appressorium. The wall adjacent to the appressorium was usually stained deeply with cotton blue. A small peg-like projection was formed beneath the appressorium and the cell wall continued to swell around this projection. The projection gradually enlarged and finally penetrated the cell wall. In older material, the remains of the appressoria appeared as diamond-shaped structures (Pl. 5, fig. 3).

In the testa the mycelium appeared to pass through cells with little difficulty, and no constrictions of the hyphae were visible where they passed from cell to cell. In the testa the mycelium moved in an indefinite direction. In the aleurone layer the mycelium was mainly intercellular but occasionally intracellular. Mycelium was intercellular in the starch cells of the endosperm. In the scutellum and embryo, mycelium was mainly intercellular, and when intracellular no obvious penetration points were visible.

The host-parasite relationships were therefore very similar to those found in wheat infected with loose smut, and in other smut infections (Batts, 1955).

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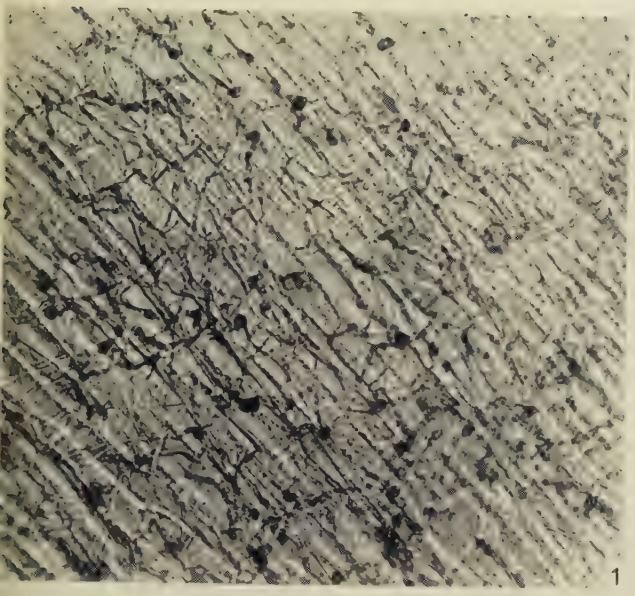
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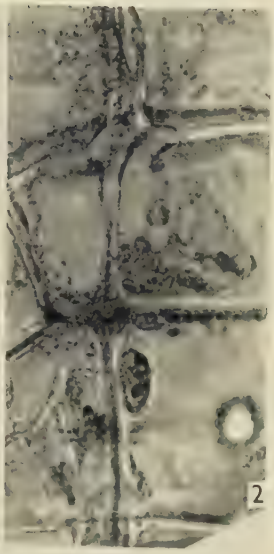
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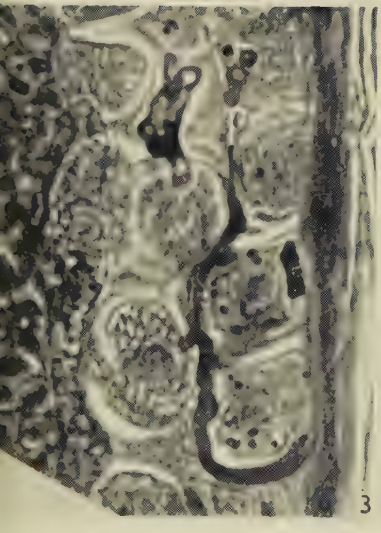
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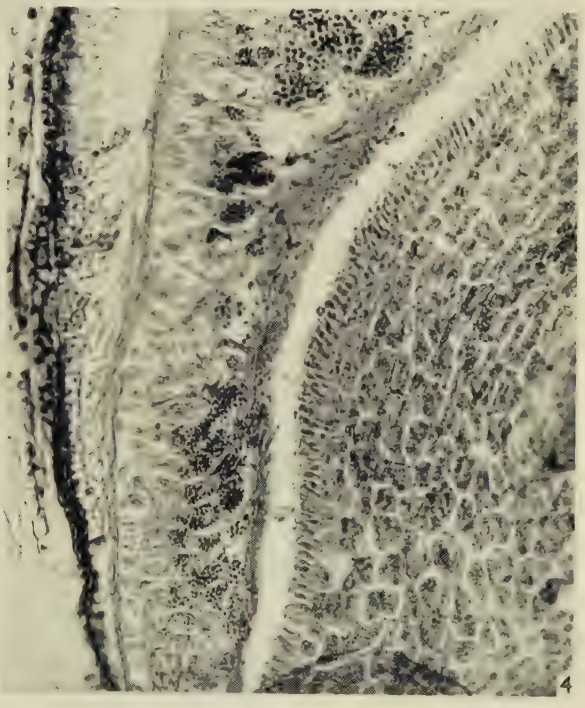
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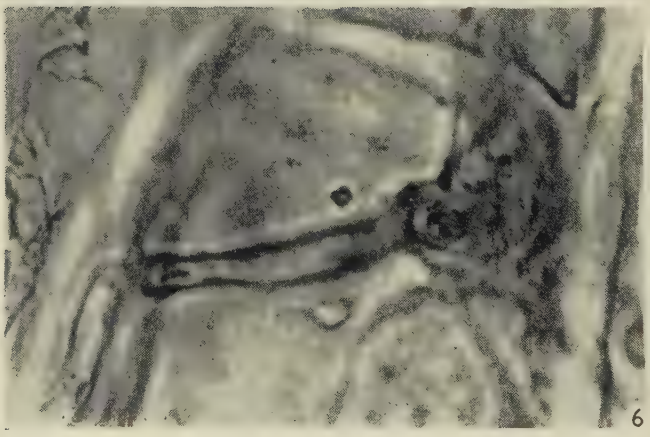
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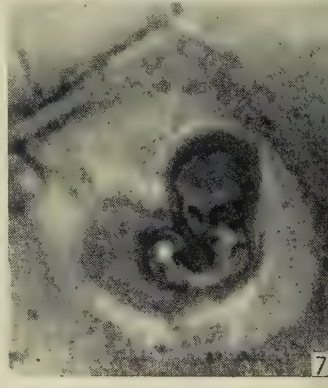
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EXPLANATION OF PLATES 5 AND 6

Plate 5

Fig. 1. Mycelium of *U. nuda* on pericarp with appressoria. × 150.
Fig. 2. Longitudinal section pericarp with intracellular hypha. × 540.
Fig. 3. Longitudinal section grain with intercellular mycelium in the aleurone. × 600.
Fig. 4. Longitudinal section grain with mycelium at the base of the endosperm and in adjacent part of scutellum. × 160.

Plate 6

Fig. 5. Longitudinal section through centre of embryo, with mycelium in scutellum and hypocotyl region. × 100.
Fig. 6. Surface preparation of pericarp with infection hyphae, 4 days after inoculation. × 1500.
Fig. 7. Surface preparation of pericarp, with appressorium and penetration point. × 1500.

(*Accepted for publication* 2 *June* 1959)

Trans. Brit. mycol. Soc. **43** (1), 126-131 (1960).

THE DEVELOPMENT OF LOOSE SMUT (*USTILAGO NUDA*) IN THE BARLEY PLANT, WITH OBSERVATIONS ON SPORE FORMATION IN NATURE AND IN CULTURE

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(With Plates 7 and 8 and 3 Text-figures)

The development of loose smut in the germinating barley seed and in the subsequent plant is the same as that previously reported in wheat. The mycelium passes with the growing point from the seed to the crown node of the seedling. It permeates the crown node, and is carried up in the young ear by elongation of the internodes of the stem. In the early stages of development of the ear, large areas are occupied by tangled hyphae, but as it develops, the mycelium aggregates in the florets. Ultimately all parts of the ear, except the rachis, are replaced by spores, produced by segmentation of the hyphae.

The fungus has been grown on artificial media and spores have been produced.

Batts & Jeater (1958) have published a detailed account of the development of loose smut in the wheat plant. The mode of infection in barley, and the production of spores in the ear, have now been investigated, and are described below.

Materials and methods

Infected seeds of the susceptible barley varieties Carlsberg and Fresia were obtained by artificially inoculating ears at anthesis by the hypodermic needle method.

For examination of the early stages of development, infected seeds were placed on damp filter-paper in Petri dishes. For examination of older plants, seed was sown in the field and also in pots. Plants were carefully lifted at different stages of growth and washed in water. About 300 infected ears, at varying stages of development, were collected. The appropriate portions were carefully removed, fixed in formalin-acetic-alcohol for 48 hr. and subsequently kept in 50 % alcohol.

The scutellum, growing region, subcrown internode, crown node, portions of internode and ears were sectioned, stained and examined as previously described (Malik & Batts, 1960). In addition, aniline gentian-violet, counterstained with Bismarck brown, was successfully used. Giemsa stain (Robinow, 1957) was used to stain nuclei.

The terminology used in describing the development of the barley ear is taken from Bonnett (1935).

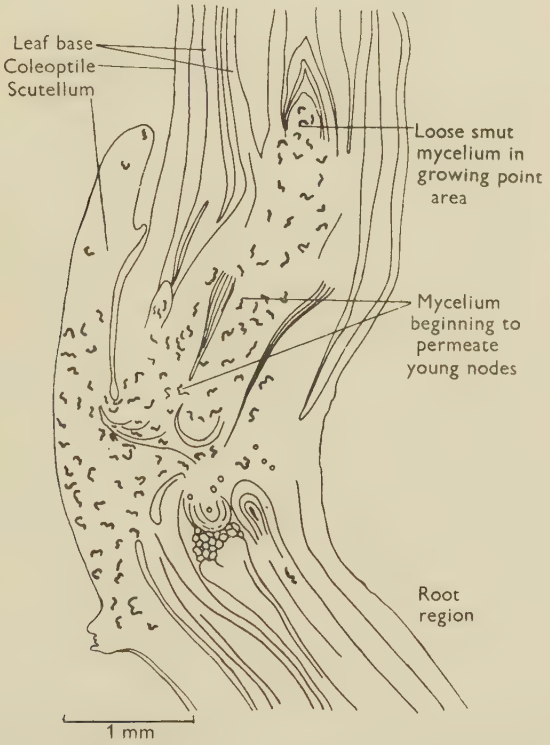
* Now at Department of Plant Protection, Block 52, Ministry of Agriculture, Karachi.

Observations

In seedlings and stems

The germination of the seed, the movement of the growing point to form the crown node near soil level, and the subsequent elongation of the stems are essentially the same as in wheat.

The position of the mycelium is also the same in the growing barley plant as in wheat (Batts & Jeater, 1958). The mycelium passes to the crown node with the growing point (Pl. 7, fig. 1; Text-fig. 1), permeates the node and so enters the growing point of the tillers (Pl. 7, fig. 2; Text-fig. 2). Infection is then carried up with the growing point and ear (as it develops) by the elongation of successive internodes, beginning from the lowest.

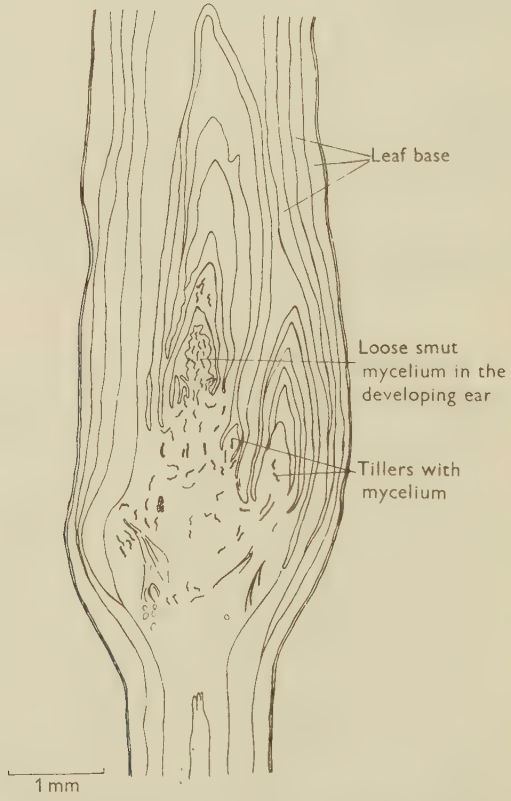


Text-fig. 1. Diagrammatic representation (longitudinal section), built up from serial sections, of the position of mycelium in a 3-week-old seedling.

The first indication of spike differentiation is the appearance of double ridges on the growing points of the tillers. The process of elongation of the rachis (axis of the ear) is similar to that of the stem. The expansion of the internodes takes place from below upwards. In the mature rachis of an infected ear, mycelium was present in each node, but none was found in the internodes (Text-fig. 3).

In the developing ear

In an ear 2 mm. long, scattered pieces of mycelium were found in the intercellular spaces. When the ear reached 5 mm., the mycelium was aggregated into small groups in the intercellular spaces, destroying the surrounding cells (Pl. 7, fig. 3; Pl. 8, fig. 4). The groups of aggregated mycelium were distributed throughout the entire ear. As the ear developed, more mycelium branched and twisted into dense tangles, and pushed the host cells apart, increasing the intercellular spaces within which spores were subsequently formed.



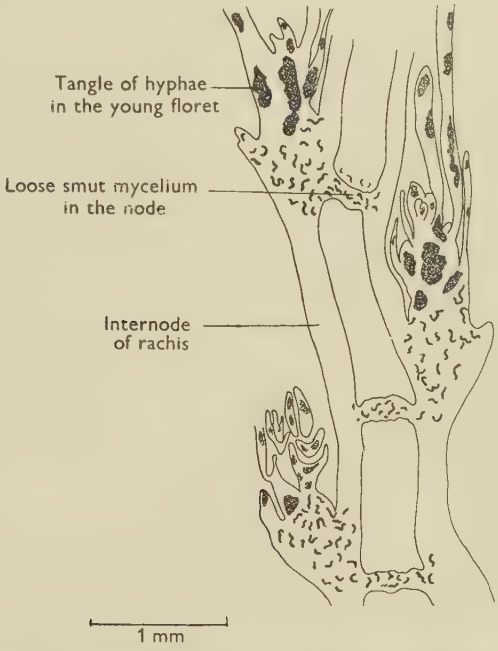
Text-fig. 2. Diagrammatic representation, built up from serial sections, of mycelium in the crown node of a 5-week-old seedling.

When the ears were 1 cm. long, large areas were occupied by the tangled hyphae and a large amount of host cell tissue was destroyed. When the ear was 2 cm. long, the colour changed from cream to light brown, this being the first macroscopic indication of spore formation.

In ears 3 cm. long, the mycelium was much branched and contorted. The hyphae were swollen and multinucleate. They massed together to form knots of hyphae of irregular shape and varying diameter, lying in a

clear matrix. The protoplasm was very dense, so that it was impossible to distinguish the nuclei. The knots of hyphae continued to enlarge and swell and the walls became completely gelatinized. In places the hyphae became more finely attenuated and broke off into segments, which at first were irregular, but as the ear developed they became round and the spore walls began to develop (Pl. 8, fig. 5).

In ears 5 cm. long, the smutted floret could easily be distinguished. The older florets in the spike contained young spore masses and usually showed different stages of spore formation, the older light-brown spores being in the centre and the young colourless spores towards the periphery; spore formation apparently originated in the centre of the knots of hyphae present in the intercellular spaces of the host.



Text-fig. 3. Diagrammatic representation of mycelium in part of the rachis of a smutted ear.

As the spike developed (measuring approximately 6 cm.) the spore masses increased and eventually coalesced, destroying the entire floret. As the spike continued to enlarge, spores were formed in increasing numbers until on emergence the ear, except for the rachis, was entirely transformed into a mass of black spores.

Discussion

Spore production by *U. nuda* has received very little attention in the literature. Wang (1934), however, showed that the spores of this fungus were formed by segmentation of sporiferous hyphae, as found in the present

study. The spores of other smut fungi, e.g. *Ustilago levis*, *U. hordei*, *U. zeae*, *U. violaceae*, *U. longissima* and *U. avenae* (Wang, 1934) and *Urocystis anemones* (Lutman, 1910), are also formed by segmentation of sporiferous hyphae.

In some other smut fungi, as in species of *Tilletia*, *Entyloma* and *Entorrhiza* for example, spores are borne singly at the tips of special short sporogenous hyphae (Fischer & Holton, 1957).

Artificial culture of *Ustilago nuda*

Appel & Riehm (1911) were probably the first to study *U. tritici* and *U. nuda* in culture. Since then, a number of workers have grown the cereal loose smut fungi on artificial media and spores have also been produced (Stakman, 1913; Sartoris, 1924; Rodenhiser, 1928; Wang, 1934; Schopfer & Blumer, 1938; Klaptsova, 1951). The smut fungi are therefore more correctly described as facultative saprophytes than as obligate parasites.

Materials and methods

Spores of barley loose smut were collected in the field and transferred directly to culture media. A few tests were also made with spores which were first suspended in 1 % copper sulphate for 24 hr. Pure cultures were then obtained by transferring the surface-sterilized spores directly to the media by means of a platinum loop.

The tests were made on the following media as given by Sartoris (1924):

(1) A nutrient solution containing: maltose, 3·0 g.; malt extract, 3·0 g.; potassium hydrogen phosphate, 1·25 g.; magnesium sulphate, 0·3 g.; peptone, 1·0 g.; distilled water, 1 litre.

(2) Nutrient agar: 25 g. agar were added to the above nutrient solution to produce a solid medium.

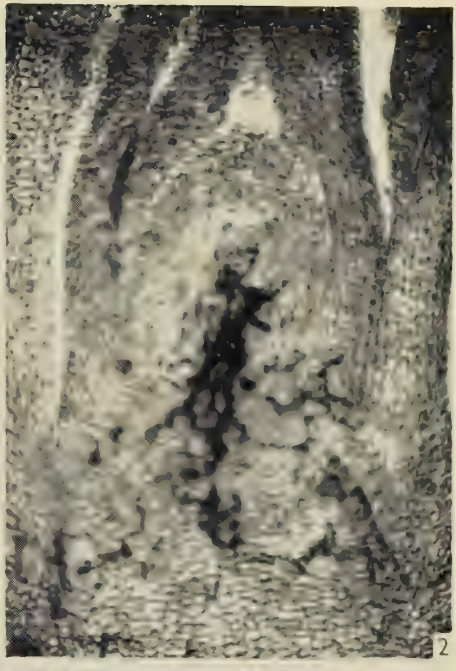
(3) Pure cultures were also grown in 5 % sucrose solution.

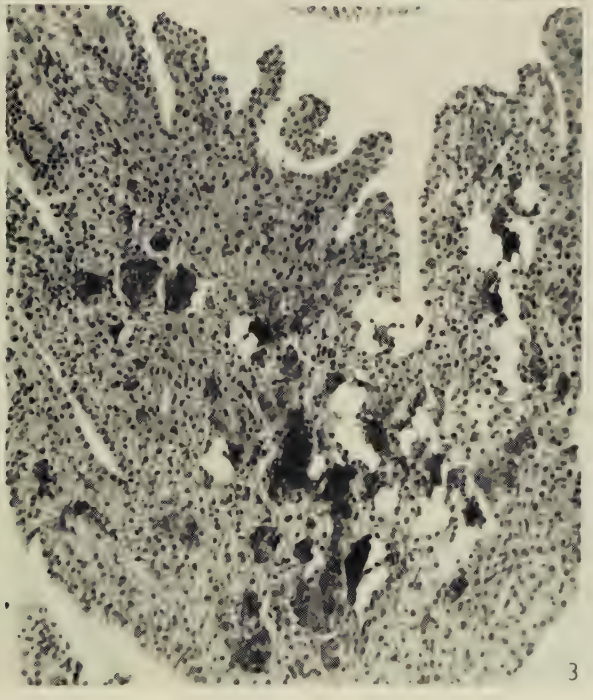
All cultures were kept at 28° C.

Observations

The behaviour of the fungus was identical in all three media. When naturally formed spores of *U. nuda* were germinated on the surface of these media, promycelia were formed in about 26 hr. Groups of spores germinated better than single spores. After about 30 hr., most spores had germinated and branches were present on the promycelia, both the promycelium and its branches being septate. After about 4 days, tiny creamy-white colonies appeared, which after about 10 days were 2 mm. in diameter. They were slightly raised, and resembled yeast colonies. After 20 days, they were 5 mm. in diameter and were slightly brown at the top. The brown mycelium was septate with irregular walls, the cells being full of protoplasm.

After about 40 days, the colony was 1 cm. in diameter, raised and dark brown in colour, and was made up of a tangle of hyphae very much resembling that observed in the host plant, the mycelium being much branched and contorted. The hyphae were somewhat swollen, multinucleate and full of protoplasm, the older filaments being empty and





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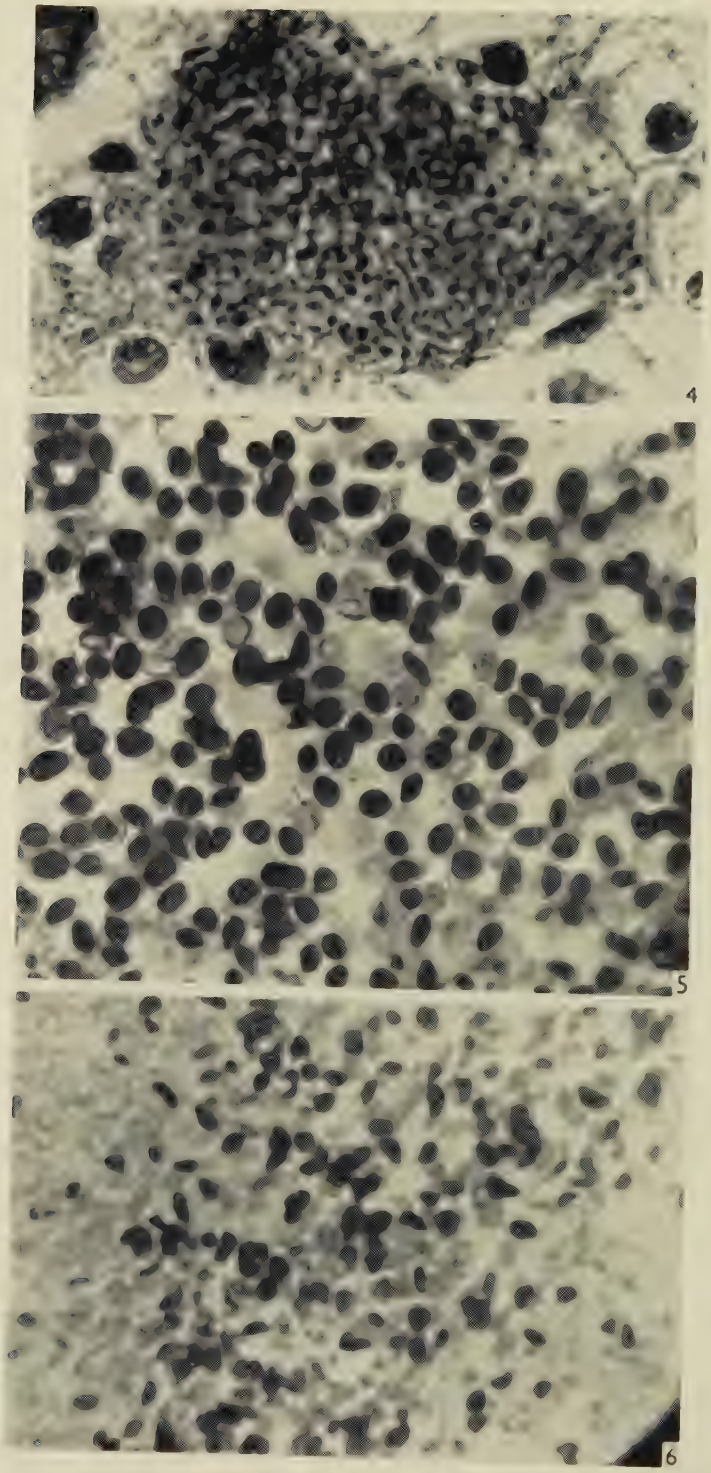
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septate. In the middle of the colony, round, dark brown bodies resembling naturally formed chlamydospores were present (Pl. 8, fig. 6). When the colony was about 2 months old, large numbers of chlamydospores were present. Some of the developing chlamydospores were obviously produced in an intercalary position, but it was not possible to ascertain whether this was always so.

Thus so far as could be determined, spores of *U. nuda* in culture were produced by segmentation of mycelium. Stakman (1913) and Klaptsova (1951) observed a similar phenomenon, but Sartoris (1924) reported the formation of spores from special branches of the promycelium.

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EXPLANATION OF PLATES 7 AND 8

Plate 7

Fig. 1. Longitudinal section, 3-week-old seedling with mycelium in growing point region. × 120.

Fig. 2. Longitudinal section, 5-week-old seedling with mycelium at the apex of the primary tiller. × 160.

Fig. 3. Longitudinal section, developing floret with pockets of aggregated mycelium. × 120.

Plate 8

Fig. 4. Longitudinal section, developing floret with spore formation in aggregated mycelium. × 1140.

Fig. 5. Longitudinal section, developing floret with maturing spores. × 1140.

Fig. 6. Section through 40-day-old colony of *U. nuda*, showing spore formation. × 900.

(*Accepted for publication 2 June* 1959)

Trans. Brit. mycol. Soc. **43**, (1), 132–145 (1960).

MYCORRHIZA IN THE GRAMINEAE

II. DEVELOPMENT IN DIFFERENT HABITATS, PARTICULARLY SAND DUNES

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(With Plate 9)

In the course of ecological studies, vesicular-arbuscular mycorrhizas were found to be widespread in the Gramineae. A quantitative method for estimating the incidence of the endophytes was devised and differences between species, habitats and communities were found. Detailed studies were carried out on a sand-dune community at Gibraltar Point, Lincolnshire; the incidence of the endophytes varied according to the colonization zone. Possible origins of infections in the pioneer, embryo-dune phase were examined. Examination of samples from two other dune systems, Southport (Lancs.) and St Cyrus (Kincardineshire), also showed that the incidence of vesicular-arbuscular infection varied with the dune succession.

Factors influencing incidence of the endophytes in sand-dune grasses are the species, degree of stability of the habitat, quantity and kind of organic matter in the sand and microbiological activity. Development of mycorrhiza may influence the part played by grasses in the dune succession. Much external mycelium and numerous penetrations (the conditions necessary for food transference to host roots) have been noted only in samples from the fixed yellow dunes. It is suggested that the association may benefit the host in this particular habitat.

Introduction

In the extensive investigations on vesicular-arbuscular mycorrhiza, few attempts have been made to estimate the incidence of root infection in different plant species or in different ecological communities. This no doubt arises from the difficulty of obtaining a quantitative assessment of infection. Methods so far devised have been used only on a restricted scale in one or a few species or habitats (Johnson, 1949; Lihnell, 1939; Mosse, 1959; Peuss, 1958). Other authorities have given the number of infected plants in different communities or associations as an indication of mycorrhizal development, but have not referred to amounts in individual species or communities (Dominik, 1951; Truszkowska, 1951).

Apparently vesicular-arbuscular mycorrhiza may be caused by a number of phycomycetous fungi. Among those recorded as causing these infections are *Endogone* spp. (Peyronel, 1924, 1937; Mosse, 1956; Dowding, 1959), species with large soil-borne spores in North America (Gerdemann, 1955; Ohms, 1956, 1957) which are thought by Dowding (1959) also to be *Endogone* symbionts, *Pythium* spp. (Hawker, Harrison, Nicholls & Ham, 1957), and isolates placed in the form genus '*Rhizophagus*'* (Barrett, 1958).

* The genus *Rhizophagus* has also been used in a more general sense to include all vesicular-arbuscular endophytes (Butler, 1939).

Owing to the difficulty of recognizing by visual means infections caused by these different fungi, there is little information on their relative prevalence in natural infections.

A previous paper described internal and external mycelial phases of vesicular-arbuscular endophytes associated with certain grasses (Nicolson, 1959). The aim of the studies reported here was to collect information regarding the distribution and intensity of such mycorrhizal infections in different species and communities, and if possible to relate these to ecology of the higher plants. While mycorrhiza in grasses is believed to be caused by *Endogone* spp. in certain instances (Nicolson, 1959), some of the infections recorded in this work may have been due to other types of vesicular-arbuscular fungi.

Methods

Estimation of root infection

A measure of the incidence of root infection was obtained, together with other data, from slides prepared in the following manner. Samples collected in the field were reduced in bulk by bunching and cutting the roots into segments of approximately 0·5–1 cm. in length. These were placed in a 1 in. layer of water in a rectangular dish, mixed thoroughly, and successively halved and quartered until a convenient quantity remained for staining. From the stained sample, segments were placed closely together in a block on a large slide (3 × 1 in.), and mounted in lactophenol under a $2\frac{1}{2}$ in. × $\frac{3}{4}$ in. coverslip. The preparation was then ringed, to give what was termed a 'root slide' (Nicolson, 1959).

Using a microscope with a mechanical stage, records were made along the entire length of a successive series of 'lines', each 2 mm. apart and parallel with the long axis of the slide. Only that part of a root segment which was crossed by the line of an ocular graticule was examined. The total number of root segments observed in this way varied with different slides, but up to 150 examinations or 'hits' were made along a line of a carefully-arranged slide. With a large coverslip, eight lines were obtained to give some 1200 root 'hits' for the entire slide.

In this way the following data were collected: (i) numbers of infected and non-infected segments were counted to give the *percentage infection* in whole, undecomposed roots; (ii) *percentage of moribund roots*, i.e. the proportion of total segments which showed varying degrees of loss of cortical cells with disintegration and decay; (iii) *percentage external mycelium*, i.e. the proportion of all roots (non-infected, infected and moribund) which showed external mycelium of vesicular-arbuscular endophytes (Nicolson, 1959); (iv) *percentage roots with brown, septate mycelium*, i.e. the proportion of all types of roots which showed the presence of brown, septate hyphae (Nicolson, 1959). Other types of fungi did not usually occur with sufficient frequency to merit quantitative estimation.

Soil estimations

Soil samples were collected in conjunction with certain root samples and the following determinations made: (i) soil reaction, as measured by

a Muirhead or Cambridge glass electrode meter, 20 g. fresh soil to 50 ml. distilled water being used; (ii) organic carbon, by Walkley & Black's Rapid Titration method, using 0·5–2 g. air-dry soil and expressed as 'Organic Carbon, Walkley & Black values' (Piper, 1947); (iii) nitrogen and calcium carbonate contents by the Kjeldahl and Rapid Titration methods, respectively (Piper, 1947).

Infection of grasses from different communities

Infections of the vesicular-arbuscular type have been noted in many grasses and cereals from different sites in Britain (Nicolson, 1955). However, these will not be listed here since little significance can be attached to records which refer only to presence or absence of infection. It is probable that the invasion of any plant (excluding those which form specialized mycorrhizas) can take place under suitable environmental conditions.

Initial observations using the root-slide technique for assessing incidence of infection were carried out to test the method, and to find whether quantitative variations existed between different samples. For this, certain local communities were chosen, particular grasses sampled and infection levels recorded (Table 1).

Table 1. *Percentage infection and external mycelium (figures in parentheses) in grasses from different habitats*

	Dactylis glomerata	*Festuca ovina*	*Deschampsia flexuosa*	*Agropyron pungens*	*Agrostis alba*
Charnwood Forest, acid heath	10	26 (6)	16	—	—
Breedon-on-the-Hill, basic	7	23 (0·4)	—	—	—
Sutton Bonington					
Cultivated plots	2 (1)	—	—	—	—
Roadside verge	10 (1)	—	—	—	—
Grass bank	0	—	—	—	—
Gibraltar Point, salt marsh	—	—	—	46 (1·5)	25 (0·7)

Considerable variation in infection was found. For instance, in *Dactylis glomerata* L. the infection varied according to the habitat and sampling site. In samples collected at Sutton Bonington, infections varied even although the sampling sites were within 100 yd. of each other. Also there was a difference in infection according to species. *Festuca ovina* L. was infected in both acid and basic soils to a greater degree than was *D. glomerata*. In acid soil conditions, *Deschampsia flexuosa* (L.) Trin. showed infection intermediate between these two species. Highest infection was noted in grasses from a salt marsh community.

The amounts of external mycelium were low in all samples (Table 1), and did not appear to be related to the amount of infection. However, this may have been due to excess washing of the root segments when samples were being drawn to make the root slides. Washing of the roots was reduced to a minimum when it was realized that it might reduce the amount of external mycelium.

The labour involved in making and taking records from root slides meant that, generally, only one root slide was made from each field sample.

However, with the *D. flexuosa* material, six root slides were made from different samples drawn off from the bulk field sample. The infection figure given represents a mean of the figures from these slides, with standard deviation 1·56. The figures for *D. glomerata* from Charnwood and Breedon were means of three slides, each with standard deviation 2·00. These low standard deviations indicated that the figures obtained from the root slides were representative of the infection levels in the bulk field samples.

Hence, it appeared that the root slide technique was suitable for assessing the relative infection levels in different grass species and in species from different habitats. Variation in infection occurred in different grasses, and in the same grass from different habitats and soil types. The intensity of infection in any one habitat, or variations noted between different habitats, did not appear to be related to any of the soil factors which were measured.

Infection of sand-dune grasses

Although differences were obtained in mature habitats, it was difficult to establish which factors were responsible for the differences in distribution and intensity of mycorrhizal infection. It was hoped that more interesting and conclusive results might be obtained by examining the grass flora from simpler types of communities, particularly where a primary succession occurred from a pioneer phase to a climax or subclimax community. Sand-dune communities were chosen as filling these requirements. The ecology of sand dunes has been extensively investigated (Salisbury, 1952) and grasses play an important part in the colonization sequence.

The sand dune where most studies were carried out is situated at Gibraltar Point, Lincs. Here samples were collected over a 3-year period. One series of samples was collected from dune systems at Southport, Lancs, and another from those at St Cyrus, Kincardineshire.

At Gibraltar Point

Variation in colonization zones

The area chosen for sampling was the most southerly section of the dunes adjoining the Bird Observatory. This area had been surveyed in detail (Barnes & King, 1952) and formed part of the Gibraltar Point Nature Reserve. The vegetation succession corresponds to the sequence found in most dune systems in this country (Tansley, 1939).

In 1952, a permanent transect line was established covering the main stages of the dune succession (Pl. 9, fig. 1). Root samples were collected in September 1952, August 1953, and June 1954, from embryo dunes, mobile yellow dunes, fixed yellow dunes and older fixed dunes. Embryo dunes and mobile yellow dunes carry unstable and changing communities, so samples were not collected from identical sites over the 3 years. Samples from fixed dunes were collected from the same sites over the sampling period. The same grasses were not always present at any one site in different years. It was therefore not possible to collect identical series of samples in the 3 successive years. Since the results obtained in each of the 3 years showed similar trends, they have been combined (Table 2).

Considerable infection and some external mycelium occurred in samples from embryo dunes, as shown in Table 2. However, in the mobile yellow phase, infection was at a minimum and external mycelium was very sparse. Highest infections and external mycelium were noted in the succeeding phase of the fixed yellow dunes. Thereafter, sampling sites were situated in closed communities, but in the first site of the older fixed dune there was only a slight reduction in infection and external mycelium, when compared with the fixed yellow dune samples. In samples from a more mature site, the figures obtained decreased considerably. Hence on either side of the mobile dune phase, there were regions of endophyte activity. This appeared to decrease progressively as maturity was developed in the fixed zones.

Table 2. *Infection and external mycelium in grasses from different colonization zones of the Gibraltar Point sand dunes*

(Inf. = percentage infection; E.M. = percentage external mycelium.)

		Agropyron junceiforme		*Ammophila arenaria*		*Festuca rubra* var. *arenaria*		*Poa pratensis*	
		Inf.	E.M.	Inf.	E.M.	Inf.	E.M.	Inf.	E.M.
Open communities	Embryo dune	14	4	15	9	—	—	—	—
	Mobile yellow	1	2	2	1	—	—	—	—
	Fixed yellow	—	—	28	37	47	20	16	20
Closed communities	Older fixed (site 1)	—	—	—	—	45	19	10	20
	Older fixed (site 2)	—	—	6	0	—	—	3	0·5

Table 3. *Moribund roots and brown, septate hyphae in grasses from different colonization zones of the Gibraltar Point sand dunes*

(M. roots = percentage of moribund roots;
B.S.H. = percentage roots with brown, septate hyphae.)

		Agropyron junceiforme		*Ammophila arenaria*		*Festuca rubra* var. *arenaria*		*Poa pratensis*	
		M. roots	B.S.H.	M. roots	B.S.H.	M. roots	B.S.H.	M. roots	B.S.H.
Open communities	Embryo dune	8	—	9	13	—	—	—	—
	Mobile yellow	5	—	2	2	—	—	—	—
	Fixed yellow	—	—	11	13	19	22	22	20
Closed communities	Older fixed (site 1)	—	—	—	—	22	12	20	18
	Older fixed (site 2)	—	—	32	—	—	—	23	27

Moribund roots and brown septate hyphae

Records of moribund roots and brown, septate hyphae were made for some of the samples collected (Table 3). Again there was an indication that the amounts varied according to the colonization sequence, in the same way as for endophyte infection and external mycelium, particularly in samples from the first three open communities. The trends in the older fixed zones were not so distinct, but the largest amounts of moribund roots and brown, septate hyphae occurred in the most mature site.

Pioneer colonization phases

One of the most surprising features with regard to the occurrence of vesicular-arbuscular endophytes in sand-dune grasses was the relatively high level of infection in samples from the pioneer embryo dune phase (Table 2). The main problem regarding these infections is the method of dissemination and spread of the endophytes to these isolated communities in more or less virgin sand (Pl. 9, fig. 2).

The possibilities are, first, that the endophytes are present in the sand prior to the arrival of the colonizing plants. Embryo dunes are initiated on the foreshore driftline where deposits of organic matter occur. This organic material at Gibraltar Point consists mainly of buried *Flustra* sp.,* but considerable amounts of plant materials are also present. It is possible that some of this arrives from older parts of the dunes, and may carry the endophyte. The second possibility is that the endophyte may be carried on the propagative units (seed or tillers) of the colonizing plants.

Table 4. *Infection and external mycelium in early pioneer colonization phases*

(Inf. = percentage infection; E.M. = percentage external mycelium)

(*a*) In field samples of *Agropyron junceiforme*

Sample	Site and community type	Infection	External mycelium
Young seedling plants (with seed still attached)	Foreshore—open community with scattered seedling plants	0	0
Young plants	Dune islands in isolation from main dune system	3	2
Mature plants	Well-formed embryo dunes	10	1
Mature plants	At base of mobile dune ridge	32	35

(*b*) In *Ammophila arenaria* grown in pots of driftline sand

		Seed		Tillers	
		Non-sterilized	Sterilized	Non-sterilized	Sterilized
Non-sterilized sand	Inf.	0·8	0·3	0·8	5·0
	E.M.	0·8	0·6	0·3	4·0
Sterilized sand	Inf.	0	0	0	0
	E.M.	0	0	0	0

To examine the possible origin of infections in embryo dune plants, two lines of investigation were carried out. First, non-gramineaceous plants restricted to the foreshore and typical salt-marsh plants on areas adjacent to the dunes were examined for infections. These included *Salsola kali* L. from the foreshore and *Salicornia stricta* Dum. (= *S. herbacea*) and *Suaeda maritima* (L.) Dum. from marsh areas. No trace of vesicular-arbuscular infection or external mycelium was observed. Hence, mycorrhiza was absent in plants arising from propagative units from parent plants already in the area and not from older dune phases.

Secondly, more extensive studies were conducted on the grasses in early colonization zones, both in the field and by means of pot tests. In 1954,

* Known as 'sea-mat', and classified with the Bryozoa.

field samples of *Agropyron junceiforme* were collected from the earliest possible stages to find out when this plant became infected during the course of its establishment on the driftline, and to trace the development of infection in pioneer dune formation prior to the mobile dune (Table 4*a*).

The results shown in Table 4*a* can be summarized and amplified as follows. No infection was observed in a seedling sample. In a sample from dune islands, the endophyte was present but in very small amounts. In well-formed embryo dunes, considerable infection occurred and at a site at the base of the mobile dune ridge, high infection and external mycelium were present (Pl. 9, fig. 2). This latter site was only 8 yd. below the crest of the mobile dune ridge where low infection was always noted (Table 2), and represented the stage at which embryo dunes were being fused to the main dune system. Hence, these samples indicated that, although *A. junceiforme* was not infected in the earliest seedling stage, infection originated in dune islands and increased considerably as embryo dunes were formed and incorporated into the main dune system.

To test the assumption that endophytes were transported to the embryo dunes on either seed or tillers of the colonizing grasses, a small pot experiment was carried out in 1954, using combinations of non-sterilized and sterilized *Ammophila arenaria* seed and tillers, respectively, grown in non-sterilized and sterilized sand. The sand was obtained from the driftline, from sites where no plants were growing. Seed and tiller bases were sterilized by immersion in 1/1000 mercuric chloride and were then washed in sterile water. Sand was sterilized by autoclaving at 20 lb. for 20 min. The results of this test are shown in Table 4*b*. Small amounts of infection and external mycelium were noted in treatment-series with non-sterile sand, but no trace of the endophytes was noted in plants grown in sterilized sand.

These observations from field and pot samples indicate that the fungi are not 'seed-borne' or 'tiller-borne' but are 'soil-borne', spreading on plant debris to the driftline sand in advance of colonizing grasses. From small amounts of initial infection, the endophytes can build up in the sand during early colonization to give appreciable levels of infection (Table 4*a*). Further evidence, however, would be necessary to establish these tentative conclusions.

Soil estimations

In all zones pH readings were high and, while the trend of increasing acidity generally found in sand dunes (Salisbury, 1952) was evident in certain series of samples, it was not so in others (Table 5). Calcium carbonate contents were also high and there was no marked decrease in the older dunes, as normally found in other dune systems (Salisbury, 1952). The figures for pH are higher than those recorded for other east coast dunes such as St Cyrus (Gimingham, 1951) and Blakeney Point (Salisbury, 1922), and are more comparable to records from alkaline types of dunes (Salisbury, 1925; Brown, 1958*b*). The high level of carbonates at Gibraltar Point may account for the high pH readings.

Very small amounts of organic carbon (expressed as Walkley & Black

values) were present, particularly in the early colonization zones (Table 5). Generally the estimates were slightly lower in the mobile dunes in comparison to embryo dunes, and in later zones there was a gradual increase. Total nitrogen followed the same general pattern. In the early zones very small amounts were present but levels were higher in the later phases, giving C/N ratios of approximately 10, which is the ratio found in most mature soils.

Table 5. *Soil determinations: Gibraltar Point*

	pH		%	%	%	Arbitrary units of organic matter content	
	24. v. 54	29. vi. 54	$CaCO_3$	organic carbon	total nitrogen	Undecomposed	Decomposed
Embryo dune	8·74	7·50	8·25	0·60	0·0098	4	1
Mobile yellow	8·08	8·20	6·25	0·46	0·0090	0	0
Fixed yellow	7·97	7·70	5·20	1·51	0·0126	1	2
Older fixed (site 1)	7·60	7·70	6·00	3·37	0·0336	3	3
Older fixed (site 2)	7·40	7·65	6·50	8·71	0·5460	2	3

The organic carbon estimates do not reflect the true amounts of organic matter in the dune sands, particularly in embryo dunes where there are considerable local deposits in the form of organic debris of the driftline. The grass roots in this zone are found to be closely associated with the debris. Organic matter is at a minimum in the surface layers of the mobile dunes, since the substrate consists chiefly of mineral sand. The increased organic matter in the older dunes is caused by deposition through colonizing plants. Attempts were made to score by eye these amounts of organic matter and the relationship between 'undecomposed' and 'decomposed' materials, using a simple flotation technique. Equal quantities of sand were shaken in equal amounts of water for 1 hr. on a mechanical shaker, and the contents of the bottles allowed to settle. The undecomposed material tended to float to the surface, whereas the decomposed colloidal material settled as a dark layer on the mineral sand (Plate 9, fig. 3). Attempts were made to designate these layers by arbitrary units (Table 5). Appreciable undecomposed organic matter was present in embryo dune sand but there was little colloidal material. Little organic matter of any kind was present in the mobile yellow dunes. With sand from the older dunes, both undecomposed and decomposed materials increased progressively, and the organic debris which floated to the surface consisted of finer fragments.

In other sand-dune communities

Examination of samples from one sand-dune community at Gibraltar Point indicated that degree of infection and amount of external mycelium varied according to the vegetational succession. To determine whether this variation was a general feature of sand dunes and not restricted to one system, samples were examined from two other sand dunes. These were situated at Southport, Lancs, and St Cyrus, Kincardineshire. On both systems, ecological studies had been carried out on the higher plant communities (Salisbury, 1925; Gimingham, 1951). The samples from South-

port were collected in May 1954, and from St Cyrus in July 1951. In both areas they were obtained from sites along a transect line traversing the dune systems. The results obtained are shown in Table 6. In general they show similar trends to those obtained at Gibraltar Point (Table 2). Variations which do occur can be related to some degree with differences in the dune sequence.

Table 6. *Infection and external mycelium in grasses from colonization zones of two sand-dune systems*

(Inf. = percentage infection; E.M. = percentage external mycelium.)

(*a*) Southport, Lancs

	Agropyron junceiforme		*Ammophila arenaria*		*Festuca rubra* var. *arenaria*	
	Inf.	E.M.	Inf.	E.M.	Inf.	E.M.
Embryo dune	15	14	—	—	—	—
Mobile yellow	—	—	1	0·5	—	—
Fixed yellow (Site 1)	—	—	23	19	—	—
Fixed yellow (Site 2)	—	—	22	35	—	—
Older fixed	—	—	13	10	11	3

(*b*) St Cyrus, Kincardineshire

	Agropyron junceiforme		*Ammophila arenaria*		*Festuca rubra* var. *arenaria*		*Arrhenatherum elatius*		*Dactylis glomerata*		*Holcus lanatus*	
	Inf.	E.M.	Inf.	E.M.	Inf.	E.M.	Inf.	E.M.	Inf.	E.M.	Inf.	E.M.
Mobile yellow	3	17	2	28	—	—	—	—	—	—	—	—
Fixed yellow	—	—	—	—	27	8	33	18	29	14	—	—
Older fixed	—	—	—	—	—	—	—	—	8	3	12	9

In the Southport samples (Table 6*a*), *Agropyron junceiforme* showed marked infection in embryo dunes as at Gibraltar Point. Again in the mobile yellow dunes infection and external mycelium were low. The fixed yellow dune phase at Southport is much more extensive than at Gibraltar Point, and consists of unstable hillocks and ridges colonized by *Ammophila arenaria*. Two samples from this zone gave similar infection levels, but external mycelium was much more abundant on the site farther inland. In samples from a site on the older fixed dunes, *A. arenaria* showed a reduced amount of external mycelium and infection, and with *Festuca rubra* var. *arenaria*, particularly, the amount of external mycelium fell to a low level.

While figures were obtained for pH, organic carbon and nitrogen at Southport, these will not be given in detail since they contribute little to the general discussion. Variation in pH ranged from 8·65 in embryo dunes to 7·60 in the older fixed phase. Organic carbon (Walkley & Black values) showed little variation in the earlier zones and were all in the vicinity of 0·04, with a slight increase (to 1·04) in the older fixed zone. A similar situation was found for nitrogen, with percentages around 0·01 in the earlier zones and a definite increase only in the older fixed zone (to 0·035).

The dune system at St Cyrus is much more compact and foreshortened than those at Southport and Gibraltar Point. Samples were collected in three zones only, as the embryo dune phase was not represented (Table 6*b*). The main feature of these results is the high amount of external mycelium recorded in the mobile yellow phase, while infection remained at levels comparable to those at Gibraltar Point and Southport. Otherwise, trends at St Cyrus were similar, with highest infection in the fixed yellow dune and amounts falling in the older dunes. The samples collected at St Cyrus were only preliminary, and it would have been interesting to have obtained a more detailed series of the more abundant grass flora, and to have extended sampling to cover the dune pasture which is represented (Gimingham, 1951).

Hence, results obtained at Southport and at St Cyrus confirmed those of Gibraltar Point. Root infection by, and external mycelium of, vesicular-arbuscular endophytes in the grass flora varied according to the dune succession.

Discussion

Three major features which may be expected to influence the distribution and intensity of vesicular-arbuscular development are: (i) the genotype of the invaded plant; (ii) the macroecological factors, particularly edaphic, which influence both fungus and higher plant; (iii) the microecological factors, particularly microbiological activity in the soil and rhizosphere. The effects of the second group are most evident and will be discussed in greater detail.

Results from mature habitats give little information on the effect of ecological factors on mycorrhizal development. However, the data from sand-dune samples show that allogenic and autogenic factors related to the dune succession do have an important influence (Tables 2 and 6). The pioneer embryo dunes are essentially a migratory type of community, but frequently they may last a number of years before conversion into the next phase. This period of relative stability would give sufficient time for build-up of the endophyte in the sand around the root systems. As previously indicated, the main problem regarding infections in embryo-dune grasses is the method of spread and dissemination of the endophytes to this isolated community (Pl. 9, fig. 2). However, from very small amounts of initial inoculum the fungi can increase considerably (Table 4). The ample aeration of the sand substrate, together with organic matter in the vicinity of roots (Pl. 9, fig. 3), would appear to stimulate the activity of the endophytes.

The main characteristic of the mobile yellow dunes is their pronounced instability. Here there is continual accumulation of sand increasing the height of the dune. The ability of *Ammophila arenaria* to keep pace with this, by rapid upward growth, gives it the adaptive advantage for almost sole occupation of this habitat. It follows that surface roots, in a practically pure mineral-sand medium, are always newly formed and there is little time for fungal build-up around them. Hence, the unstable qualities of this community, together with the low organic matter content, would appear to be the chief factors responsible for the low infections and small

amount of external mycelium in this zone. The smaller amount of root decay and the low figures for the mycelium of other fungi on the roots (Table 3) indicate that general microbiological activity is also low.

The frequent infections and abundant external mycelium recorded in the fixed yellow dunes indicate that in this zone conditions are optimum for endophyte development. Here there is little sand movement and the surface is relatively stable. Hence there is no continual development of new roots in the surface layers, and this would give the endophytes an opportunity to build up around the grass roots. Organic matter resulting from the decay of colonizing plants is beginning to increase (Table 5), but apparently not in amounts sufficient to stimulate microbiological activity to an extent that would have an antagonistic effect on the endophytes.

As previously shown, there was some indication in samples from mature habitats of varying mycorrhizal intensity in different grass species (Table 1). However, it was in the fixed yellow dunes at Gibraltar Point, where the range of three grasses overlapped, with roots actually intermingling in the sand, that this difference was most marked. Here, *Festuca rubra* var. *arenaria* showed consistently high infection and *Poa pratensis* showed lowest infection, with *Ammophila arenaria* intermediate (Table 2). The amounts of external mycelium did not appear to be related to root infection. Highest amounts occurred on *A. arenaria* roots, with only half the amount in the other two species. However, amounts of external mycelium may be affected by washing and by the nature of the roots.

Variations in mycorrhizal intensity in older colonization zones at Gibraltar Point are more difficult to explain, but again the stability of the habitat and the organic matter content would appear to be involved. High infection can still be maintained after the community has become closed, indicating that there was little difference in endophyte activity in the yellow fixed dune with an open community and the early phases of the closed community. However, where the dunes are older there was a considerable reduction in infection (Table 2). In this stage of the dune succession the organic matter, particularly colloidal material, is increased (Table 5) and this will stimulate greater microbiological activity.

No microbiological determinations on rhizosphere or soil were carried out in conjunction with the studies on mycorrhiza of sand-dune grasses, although there is ample evidence that development of a sand-dune microflora is associated with the vegetational succession (Webley, Eastwood & Gimingham, 1952; Brown, 1958*a*, *b*). Webley *et al.*, using a dilution plate technique, demonstrated that numbers of both bacteria and fungi markedly increased with initiation of plant colonization and rose steadily with vegetation development. Likewise, Brown (1958*a*), using a direct, soil-impression technique, showed that amounts of fungal mycelium increased progressively up to the fixed-dune phase.

Hence it can be said that fluctuations in vesicular-arbuscular mycorrhiza in grasses from colonization zones of sand dunes are due to a combination of factors. While there may be variation with species the chief influencing factors are considered to be, in early zones, the degree of stability of the habitat together with the amounts of 'undecomposed' organic matter present; and in later zones, increase in 'colloidal' organic matter, resulting

in greater microbiological activity. Changes in other edaphic factors measured, e.g. pH and calcium carbonate, did not appear to influence directly the incidence of the endophytes. These conclusions are based on field evidence, and confirmation by experimentation would be desirable.

Both Webley *et al.* (1952) and Brown (1958*b*) suggest that the microflora of dune soils may influence the higher plant succession. In particular, the former workers state: 'There can be little doubt that the activity of soil micro-organisms contributes to the maturation of the habitat, and therefore constitutes a biotic factor adding its influence to the other more familiar factors causing change in plant communities.' It has been shown that development of vesicular-arbuscular mycorrhiza is likewise correlated with the dune succession, and it may be regarded as an additional factor influencing colonization of sand dunes by the grass flora.

From an examination of the three fungal phases it seems that a physical channel for food transference might exist when abundant external mycelium and numerous penetrations of roots are present (Nicolson, 1959). However, from samples already examined it would appear that these required conditions do not occur frequently in nature. A high incidence of external mycelium has been noted only in grass species from the fixed yellow zone of sand dunes. It is likely that this transitional phase is the most exacting of the dune habitats, with the roots of many plants in competition with one another, and with an active soil microflora, for nutrients in a limited volume of sand. It is conceivable that, in this particular habitat, the development of frequent root infection with extensive external and penetration phases may benefit the invaded plants. But in the preceding yellow mobile dune, *A. arenaria* is completely dominant and can tap the entire nutrient resources of the habitat by means of an extensive root system traversing a large volume of sand; here microbiological activity seems to be at a minimum. In the succeeding mature habitats of the older fixed dunes a 'soil' is being formed, enriched by accumulation of plant remains from a closed community, and with a more liberal supply of available nutrients.

It is hoped that this work on the primary succession of sand dunes may provide a basis for examination of grasses in more complex and mature communities, and eventually under conditions of cultivation. Recent work indicates that vesicular-arbuscular mycorrhizas may sometimes benefit the invaded plant (Hawker & Ham, 1957; Mosse, 1957; Peuss, 1958; Winter & Meloh, 1958). If this does occur, even to the smallest degree under certain ecological conditions, the importance of the phenomenon cannot be minimized in view of its world-wide distribution in the Gramineae and in other families of plants.

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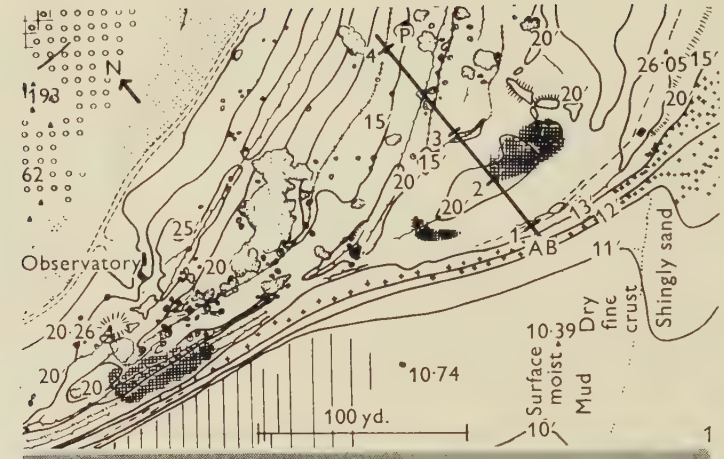
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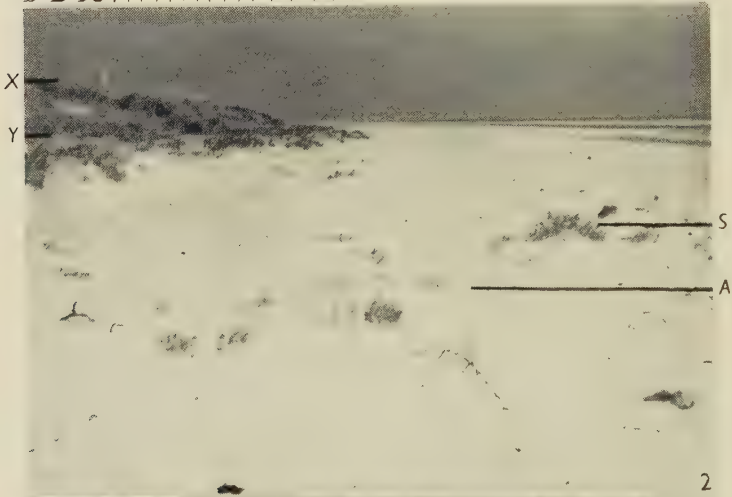
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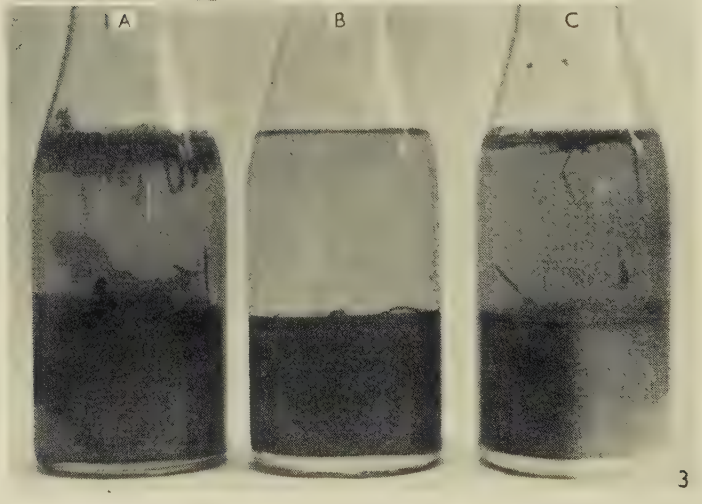
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EXPLANATION OF PLATE 9

Fig. 1. Base map of section of dunes at Gibraltar Point where samples were taken. (After Barnes & King, 1952. Reproduced by kind permission of the Lincolnshire Naturalists' Trust, Ltd.) The approximate locations of the sampling sites are shown on the transect line *AB*: 1, in mobile yellow zone; 2, in fixed yellow zone; 3, in older fixed zone (site 1); 4, in older fixed zone (site 2).

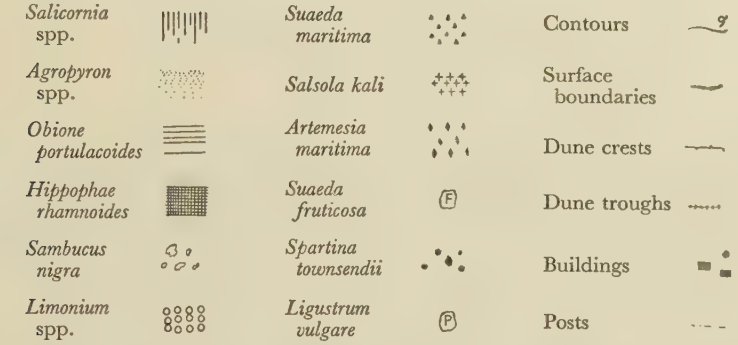

Fig. 2. View showing the foreshore driftline, embryo dunes and mobile yellow dune ridge at Gibraltar Point. *A*, *Agropyron junceiforme* forming a young embryo dune, *Salsola kali* (*S*) is also present; *X*, summit of the yellow dune ridge where low incidence of vesicular-arbuscular infection occurred; *Y*, base of the yellow dune ridge where appreciable infection occurred (see Table 4).

Fig. 3. Method used to obtain separation by flotation of organic matter in dune sands. The 'undecomposed' material tended to float to the surface of the water and the 'decomposed' to settle on the sand surface. A, from embryo dunes; B, from mobile yellow dunes; C, from fixed yellow dunes.

(*Accepted for publication* 1 *August* 1959)

Trans. Brit. mycol. Soc. **43** (1), 146–149 (1960).

EARLY GROWTH STAGES OF *NEUROSPORA CRASSA*

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(With 1 Text-figure)

When freshly formed spores of *Neurospora crassa* are plated on nutrient agar and allowed to grow within the temperature range 19–27° C., the area of the mycelial mat, as a function of time after germination, can be represented in the first 24 hr. by three linear segments containing between them two sudden changes of growth rate. It is suggested that these changes may be determined by diffusion from the spore surface of substances affecting growth.

Observations made during the course of some other work (Rosen, 1957) led us to search for evidence of a sudden change in the growth behaviour of *Neurospora crassa* in the first 15–20 hr. after fresh spores had been plated out on a nutrient agar gel. The purpose of this note is to demonstrate that, in fact, there are two such sudden changes.

We examined the fungal growth by determining the change with time of the average diameter of the mycelial mat. This method, or one or other variation of it, has been used in classical studies of the growth of various fungi (Fawcett, 1921; Brown, 1923) and of *N. crassa* in particular (Ryan, Beadle & Tatum, 1943). But in these and such other related studies as we have found (e.g. Emerson, 1950), measurement has been made either of growth rate after 24 hr., when it has become steady, or of mycelial size at some fixed time after plating, regardless of the intervening growth rate. We have found no record referring to the dynamics of mycelial growth in the first 24 hr. after plating.

Method and results

In all experiments we used *N. crassa* of Commonwealth Mycological Institute strain no. I.M.I. 53238 and Fries no. 3 nutrient medium in a gel of 4 % washed agar, following the recommendation of Ryan *et al.* (1943) both in the medium used and in treatment of the agar. A layer of gel 2–3 mm. deep in a Petri dish of diameter 3·5 cm. was centrally inoculated with spores on the point of a platinum needle, with care taken that the inoculum covered a minimal area. The spores were freshly formed ones, appearing 2 days after parent colonies had been plated in similar dishes. The size of a colony was determined by examining the inverted dish under a microscope with a calibrated scale in the eyepiece. Between measurements, which lasted a few minutes, the dish was kept in a thermostat.

For each measurement, the mean diameter was obtained from six readings and thence a value for the area of the mycelium was determined, together with the standard deviation of that value. Measurements were

made at intervals of 50–70 min. After 24 hr. measurements were discontinued, since Ryan *et al.* (1943) report that the growth rate thereafter is constant, and because the growth rate we recorded had by then approached that given by Ryan *et al.*, allowing for some strain difference.

In Fig. 1 are plotted the areas against time, for two colonies at different temperatures.

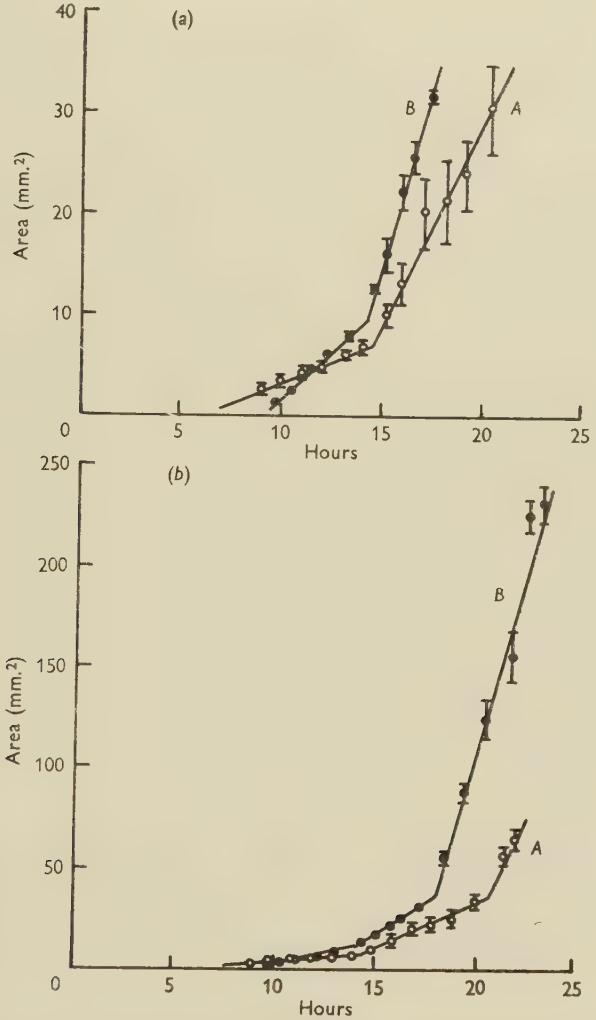

Fig. 1. Area of mycelial mat of *N. crassa* as a function of time after plating spores. *A*, colony grown at 20·0° C.; *B*, colony grown at 26·5° C. (*b*) shows the same colonies as (*a*), but with a contracted ordinate scale.

Over the limited temperature-range within which we worked (19–27° C.), we found that the first change in growth rate occurred at $14{\cdot}5 \pm 0{\cdot}3$ hr. after plating, despite a 30% difference in area reached, whereas the second change occurred when the area of the colony was 36 ± 3 mm.² (radius

3·38 ± 0·14 mm.) despite a 30 % difference in time taken to reach this size (Fig. 1). These features of the growth were such that at higher temperatures the two changes approached so closely in time that their occurrence was not determinable, whereas at lower temperatures the early growth was so slow that the straight lines were of little statistical validity.

Discussion

Various answers have been given to the question of whether mycelial growth is most properly followed by radius, area, differential area, dry weight, or some other property (e.g. Worley, 1939; Hawker, 1950; Braccanto & Golding, 1953). Evidently a graph against time which is linear for area will be convex for radius and concave for weight, and *mutatis mutandis* for other linear graphs. We have plotted the variable which best demonstrates our point that there are sudden changes in growth behaviour. From Fig. 1 it can be seen that, during the first 20 hr. after germination of the spores, the area covered by the mycelium can be accurately represented by three linear segments containing between them two sharp changes in growth rate. Although the straight lines drawn in Fig. 1 exaggerate the effect, it appears that the changes in growth rate take place over a period of 30–50 min.

As an exclusive list of factors which separately or together might cause sudden changes in growth rate we may take: escape from the action of an external growth-inhibiting agent; response to an external growth-stimulating agent; and suddenly occurring internal changes in metabolism, physiology, etc. The first of these factors is of particular interest in regard to the possibility of growth-inhibiting substances, on the surface of the spores, which would diffuse out into the gel after plating. It is possible that either or both of the breaks in the growth curve may be determined by the diffusion from the spore surface of substances affecting growth.

We have not observed any morphological features associated with either of the two breaks in the growth curve. Septa and aerial shoots both appear within 10 hr. of plating.

This work has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Institutes of Health, U.S. Public Health Service.

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(*Accepted for publication* 1 *August* 1959)

Trans. Brit. mycol. Soc. **43** (1), 150–154 (1960).

PHYSIOLOGIC SPECIALIZATION IN *POLYSPORA LINI* LAFF.

By JOHN COLHOUN

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Experiments made over a 4-year period have demonstrated that variability in *Polyspora lini* Laff. occurred when the reactions of the flax varieties Blenda, Herkules, Norfolk Earl and Norfolk Queen to stem-break were considered. None of the varieties tested was immune to one race of *P. lini* and susceptible to another in the stem-break phase, so that the separation of races is based on the level of attacks on the differential varieties. It is difficult, if not impossible, to retain isolates growing in artificial media for a year or longer without alterations occurring in the level of attack on at least some of the differential varieties.

It has been demonstrated by Henry (1934) that in culture *Polyspora lini* Laff. may produce saltants in the form of sectors which differ greatly in cultural characters. Henry's preliminary pathogenicity tests also indicated that the same flax variety may differ in its reaction to various strains of the fungus.

The development of a pot technique for testing the resistance of flax plants to the stem-break phase of the disease caused by *P. lini* (Colhoun, 1959) renders it possible to obtain, for any population, accurate values for the percentage number of plants susceptible to this phase. It therefore seemed possible that this technique might be employed in an attempt to establish whether physiologic races of *P. lini* exist, as this information is of value when efforts are being made to breed flax varieties resistant to this fungus.

Methods

Isolations of *P. lini* were made from flax seeds grown in different areas in Northern Ireland, England, Belgium and Holland, single-spore cultures being prepared. The fungus was maintained in culture (on 2 % malt agar) for the shortest possible period before being used for the inoculation of plants on the first occasion. When an isolate of the same origin was used in successive years, a single-spore culture was prepared in each year shortly before inoculations were made. Sectors appeared in many cultures, but these were avoided when transfers were being made. The pot technique already referred to was employed in testing the resistance of a range of fibre and oil flax varieties to each isolate, consideration being given mainly to the stem-break phase. The seed of each variety used was the product of a single-plant culture. In each experiment, all the inoculations were made on the same day and all the experimental plants involved were maintained under similar conditions, remaining in the open air except during the period of incubation after inoculation.

Results

In 1954 eight isolates of *P. lini* (A–H) entered into an experiment, the origin of each isolate being stated in Table 1. Each isolate was used to inoculate the plants in one pot of each flax variety, the eighteen varieties or selections listed in Table 2 being employed. The results obtained (Table 2) indicate that Rio (C.I. 280) was immune, whereas La Plata (selection NB) showed considerable resistance to all isolates. With some varieties, such as

Table 1. *Origin of isolates of* Polyspora lini *employed in experiments*

Isolate	Country of origin of seed from which isolated	Year in which isolated
A	Belgium	1953
B	Belgium	1953
C	Northern Ireland	1953
D	England	1953
E	Northern Ireland	1954
F	Northern Ireland	1954
G	Belgium	1954
H	Belgium	1954
J	Holland	1955
K	Northern Ireland	1955
L	Northern Ireland	1955
M	Northern Ireland	1955
N	Northern Ireland	1955

Table 2. *Variability in* Polyspora lini *in* 1954 (*growing period 25 June–6 October*)

(Mean % plants developing stem-break)

Variety or selection of flax	Isolate of *P. lini*							
	A	B	C	D	E	F	G	H
Blenda	28	40	90	71	93	72	74	77
Concurrent	100	100	94	75	84	93	98	100
Giza Purple	70	72	94	71	69	72	91	60
Herkules	24	70	55	83	72	66	63	57
Linkopis	57	59	84	94	82	72	81	73
Norfolk Earl	62	68	71	88	49	20	36	53
Norfolk Queen	12	63	42	82	93	49	31	56
Stormont Gossamer	91	92	100	100	100	91	97	97
Textilshchik	50	58	97	83	88	74	84	70
Bison	90	97	90	96	97	94	98	98
Bison (C.I. 389)	87	94	92	88	71	83	100	95
Bombay	97	100	100	100	98	98	100	98
La Plata selection BV	95	100	100	95	94	98	100	96
La Plata selection NB	14	7	1	7	2	4	19	31
Ottawa 770B (C.I. 355)	66	78	68	85	73	76	90	90
Redwing	94	100	97	100	96	85	86	100
Rio (C.I. 280)	0	0	0	0	0	0	0	0
Winona (C.I. 179)	80	93	76	91	79	88	95	93

Bombay and Stormont Gossamer, there was no variation in the percentage number of plants susceptible to the different isolates but with other varieties, such as Blenda and Norfolk Queen, some isolates brought about more severe attacks than others. Enough data were not available, however,

for the significance of these differences to be determined by statistical methods. The results suggest that fewer plants of Blenda, Herkules and Norfolk Queen developed stem-break when inoculated with isolate A than when isolates D or E were employed. A number of other varieties was very susceptible to each of these three isolates.

In 1955, an experiment was made using six isolates which had been employed in 1954. Only the five varieties (Table 3) which had been associated with high variability in the incidence of stem-break in 1954 entered into the experiment. Two pots of each variety constituted a unit. The results obtained (Table 3) show that, as in the 1954 experiment, fewer plants of Blenda, Herkules and Norfolk Queen developed stem-break when inoculated with isolate A than when isolates D or E were used. A severe attack did not occur on plants of any variety inoculated with isolate A.

Table 3. *Variability in* Polyspora lini *in* 1955 (*growing period* 3 *May–*18 *August*)

(Mean % plants developing stem-break.)

Variety of flax	Isolate of *P. lini*					
	A	B	D	E	F	G
Blenda	37	54	96	77	64	68
Herkules	9	11	60	41	46	39
La Plata selection NB	7	11	16	9	6	4
Norfolk Earl	31	33	100	96	89	69
Norfolk Queen	3	10	38	33	22	22

Table 4. *Variability in* Polyspora lini *in* 1955 (*growing period* 25 *April–*13 *August*) *and* 1956 (*growing period* 2 *May–*2 *October*)

(Mean % plants developing stem-break)

Variety of flax	Isolate of *P. lini*						
	C	H	J	K	L	M	N
	In **1955**						
Blenda	60·5	50·2	39·9	30·5	54·3	40·6	27·3
Herkules	56·5	36·9	41·1	61·7	60·9	51·8	25·8
La Plata selection NB	21·2	4·2	13·6	5·2	5·9	8·5	7·5
Norfolk Earl	51·5	27·3	17·3	47·8	40·0	37·8	4·8
Norfolk Queen	41·8	11·0	9·8	24·2	32·2	34·2	1·1
	In **1956**						
Blenda	47·5	8·2	43·9	25·7	39·3	32·7	4·1
Herkules	17·0	1·5	8·1	5·9	33·2	16·6	0
La Plata selection NB	2·3	16·9	12·1	5·5	3·7	4·9	4·5
Norfolk Earl	51·7	5·4	47·7	23·4	25·9	8·1	3·7
Norfolk Queen	32·7	6·3	11·1	12·6	31·4	10·2	2·4

L.S.D. for 1955, 16·8; for 1956, 15·3.

Further experiments were made in 1955 and 1956 using the five varieties included in the previous experiment, these being inoculated with seven isolates, none of which except C and H had been previously employed. Four pots of each variety constituted a unit. The results are presented in Table 4. The data for the percentage number of plants developing stem-break have

been analysed statistically; in analyses of variance, very significant effects ($P < 0{\cdot}001$) due to variety of flax and to isolate of the pathogen were demonstrated in each year, while the interaction effects were also significant (1955, $P < 0{\cdot}01$; 1956, $P < 0{\cdot}001$).

In separating races of the fungus, two isolates may be distinguished as separate races when they are associated with a significant difference between the values for percentage number of diseased plants in at least one variety of flax provided that the two isolates exhibit very similar levels of a severe attack on at least one other variety. The latter requirement ensures that isolates are not distinguished as races when one isolate brings about a lower level of attack than another on all varieties. On this basis it was possible, in 1955, to distinguish isolates C, J and K as constituting three distinct races. Isolate C (race 1) brought about a heavier attack than isolate J (race 2) on Blenda, Norfolk Earl and Norfolk Queen, although similar levels of attack resulted on Herkules. Isolate C also brought about a heavier attack than isolate K (race 3) on Blenda and Norfolk Queen, the attack of both races on Herkules and Norfolk Earl being equally severe. Races 2 and 3 can be further distinguished by the latter attacking Herkules and Norfolk Earl more severely than the former, while Blenda was attacked by both to the same extent. In the 1956 experiment, isolate C brought about a heavier attack than isolate J on Norfolk Queen, while each attacked Blenda and Norfolk Earl to similar extents. It must, however, be remembered that in 1956 isolate K brought about a low level of attack on all varieties.

Discussion

Four years of study have demonstrated the difficulties experienced in obtaining identical results in successive years when isolates of *P. lini* of the same origin are employed for inoculating various flax varieties. With some isolates, the level of attack in the stem-break phase on certain varieties declined when the isolate was maintained in culture for periods of a year or longer, but it cannot be concluded that the pathogenicity of all isolates declines with age. Such differences in the level of attack as have been observed when an isolate was used for inoculating the same variety in successive years may probably be due to saltation. The results obtained indicate that it is very difficult, if not impossible, to retain isolates on culture media without some alteration in pathogenicity.

In field experiments made by Baylis (1941) in New Zealand, the variety Rio was found to be immune to the browning phase of the disease on both leaves and stems, although microscopic examination showed that ripe sepals and capsules could be attacked. In the present study, Rio has remained immune to stem-break in all experiments. None of the flax varieties tested during the course of the work was found to be immune to one or more races of *P. lini* and susceptible to others. When stem-break is considered, the results obtained show that certain varieties may be more susceptible to one isolate of *P. lini* than to another of different origin, and this observation demonstrates the existence of physiologic specialization in this fungus. The contrast in levels of attack following inoculation with different isolates is well illustrated by the results obtained from an experiment made in 1955

(Table 4). These results are supported by the data obtained in other years.

The occurrence of physiologic races in *P. lini* emphasizes the necessity for employing isolates of different origins when testing plants for resistance, and it is also desirable that the isolates employed should be grown on culture media for the shortest possible periods.

In some of the varieties employed during the investigation, a certain proportion of the plants was resistant to an isolate of *P. lini* and the remainder were susceptible. This contrast between immunity and susceptibility of plants within a variety, even when this constitutes a single plant culture, may be regarded as indicative of heterozygosity for resistance in the seed sample used. Results of a similar nature have been obtained by Macfarlane (1955) and Ayers (1957) when investigating the occurrence of physiologic specialization in *Plasmodiophora brassicae* Woron., various crucifers being used as race differentials.

The writer wishes to express his thanks to Prof. A. E. Muskett for his interest in the work, to Mr E. V. B. Wilson for supplying seed samples and to Mr G. I. Janeway for technical assistance.

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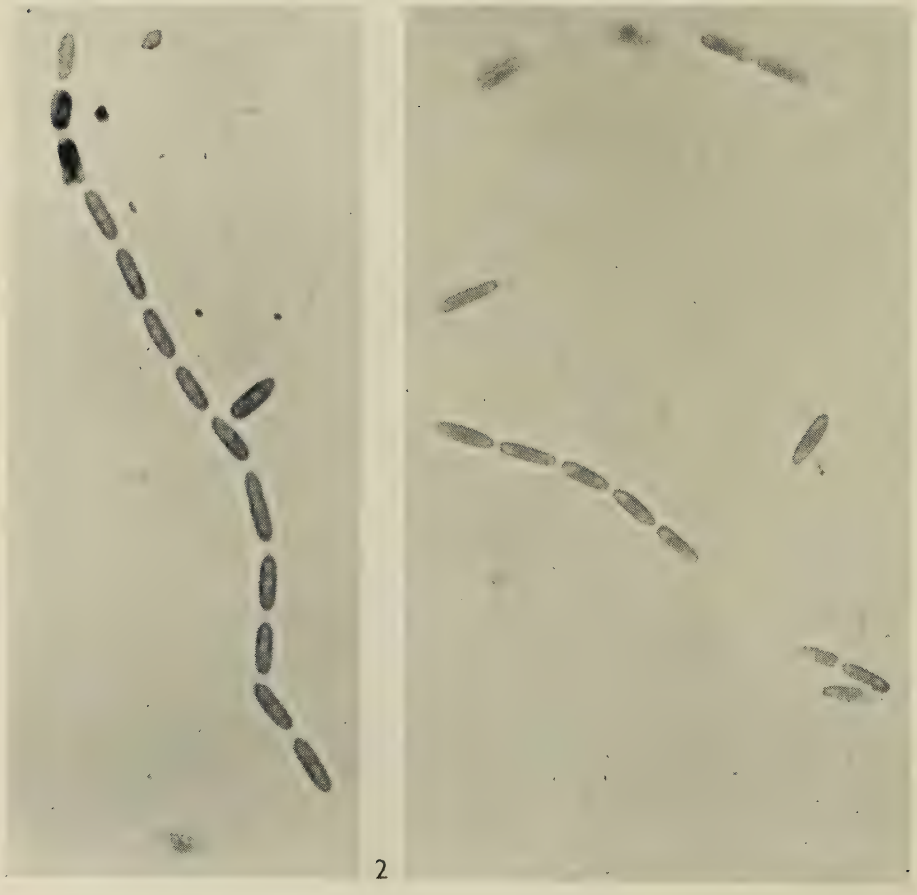
(*Accepted for publication* 8 *June* 1959)



(*Facing p.* 155)

Trans. Brit. mycol. Soc. **43** (1), 155 (1960).

BRITISH RECORDS, 42

(With Plate 10)

Paecilomyces betae (Delacroix) Cornford comb.nov. (*Oospora betae* Delacr. *Bull. Soc. mycol. Fr.*, **13**, 116, 1897) was found on the dried stems of wild and sugar beet (*Beta vulgaris*) at Dunholme, Lincs. on 17 February 1958. The stems were those on which *Pleospora betae* was also found (C. E. Cornford, *Plant Path.* **7**, 113, 1958). Pieces of stem were placed in damp air. After one or two days white, cottony chains of one-celled, hyaline conidia measuring $14–16 \times 4\,\mu$, were produced from the tips of slender conidiophores (Pl. 10, Figs. 1 and 2). The fungus often grew upon the perithecia of *P. betae*. It spored readily on sterilized sugar beet seed balls, wild beet stems and potato dextrose agar, where it formed concentric rings of sporing zones when exposed to the alternation of daylight and darkness, and a more or less uniform surface when placed in continuous darkness.

The first known record of *Paecilomyces betae* is that from France, where it was found in the winter on rotting tap roots of cultivated beet (Delacroix, 1897).

It has been reported from the Ukraine on stored beet seed (Borisiewitch, *Zbl. Zuckerind.* **39**, no. 5, 120, 1931). *Oospora* sp., which is probably the same fungus, was reported on rotting tap roots of sugar beet in Czechoslovakia (F. Rambousek and F. Neuwirth, *Z. Zuckerind, Prague*, **40**, 53–60, 1930) and on beet in Spain (F. Dominguez Garcia-Tejero, *Bol. Pat. Veg. Ent. agric., Madrid*, **18**, 181–204, 1951).

Specimens have been deposited at the Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey. (Herb. I.M.I. Nos. 72674, 72049*a*).

Thanks are due to Mr C. Booth of the Commonwealth Mycological Institute for identifying the fungus, and to Mr V. Stansfield for Pl. 10, figs. 1 and 2.

C. E. CORNFORD, *Dunholme Field Station, Lincs.*

EXPLANATION OF PLATE 10

Paecilomyces betae

Fig. 1. Conidiophores.
Fig. 2. Chains of conidia.

(*Accepted for publication* 16 *April* 1959)

REVIEWS

The Biology of Mycorrhiza. By J. L. Harley. (London: Leonard Hill, 1959.) Pp. xiv + 233, 11 plates, 15 text-figures. Price 55*s*.

Few subjects have a more extensive or more confusing literature than that of mycorrhiza; few have suffered from such divided opinions held by such uncompromising protagonists. Dr Harley has produced a reasoned and enjoyable account of this diverse field, conveying to the reader his personal viewpoint and the excitement of the subject without deviating from a balanced and scientific treatment.

The book divides into three parts. The first contains a general introduction and a chapter on the association between micro-organisms and the roots of higher plants. This chapter is decidedly selective but provides a useful background against which the special case of the mycorrhizal association may be viewed.

The second section of the book deals with the ectotrophic mycorrhiza of forest trees. This is the field in which Dr Harley is expert, and the chapters show a remarkably balanced point of view, embracing the best of the classical and modern work and seeking to incorporate into a reasonable framework some of the more extreme points of view of past and present. The chapter on the ecology of mycorrhiza is included here because of its obvious importance for forest trees, but it is a pity that this chapter could not have included the endotrophic mycorrhizas and a broad view of the ecology of mycorrhiza, about which much has been written. The section of this chapter dealing with mycorrhizas and forestry is academically satisfying but a few references to sylvicultural experimental work on the establishment of seedlings would have made it seem more real to the practical man.

The third section of the book deals with endotrophic mycorrhizas, the aspect of the mycorrhizal relationship which has given rise to most controversy and special pleading. In a series of lucid chapters, and using the work on the Ericaceae and Orchidaceae as a point of reference, Dr Harley succeeds in bringing order to the chaotic literature and displays the fallacies and underlines the known and well understood facts.

The book is as valuable for what is left out as for what is put in. This selection of material backed by a very thorough treatment of the aspects thought to be important distinguishes it from most other writings on mycorrhiza and its patent success should encourage us all to follow similar methods when teaching and writing. One other valuable feature of the book is the clear statement that certain aspects of the subject need further work. This will be a source book on mycorrhiza for many generations of research workers, as well as being an excellent review for non-specialists, and is a landmark in the subject. I do not remember reading any botanical book with greater pleasure or greater profit.

N. F. ROBERTSON

Physiology of fungi. By Vincent W. Cochrane. (New York: John Wiley and Sons; London: Chapman and Hall, 1958). Pp. vii + 524, 75 figures. Price: 78*s*.

There are only two features of this book which I am disposed to criticize. One is its title: the predominating flavour is biochemical rather than physiological. This is inevitable and desirable, a natural consequence of modern research, but it would be more informative to the potential reader if the content of this book were more accurately reflected in the title. My second objection is to the inclusion of the actinomycetes with the fungi. The book gains little from this and it is undesirable in many ways to obscure the fact that the real relationships of the actinomycetes, whether biochemical or morphological, are with the bacteria.

But these are minor criticisms. Undoubtedly, this is a very good book, full of carefully arranged information. All aspects of growth, nutrition and metabolism, reproduction, responses to physical components of the environment and fungicidal action are dealt

with exhaustively from the biochemical point of view. If one is to appreciate the book fully, some knowledge of modern biochemistry is necessary; but it is so written, with no more than the irreducible minimum of biochemical jargon, that those of us whose biochemical knowledge is restricted can find it possible to read it with understanding and enlightenment. If Dr Cochrane can produce a revised edition every five years or so, to keep it as fresh and up-to-date as it is now, this book should be a valuable (if expensive!) feature of every physiologically inclined mycologist's bookshelf for many years to come. Perhaps I should add that though it is a highly priced book, it is worth it.

P. W. BRIAN

The Cellular Slime Molds. By JOHN TYLER BONNER. (Princeton University Press, 1959. [Investigations in the Biological Sciences, no. 1.]) Pp. 150, 8 plates, 13 text-figures. Price: 32*s*.

In these days of rapid scientific expansion it is refreshing to find a book which can justifiably claim to contain a comprehensive and readable survey of all work done before 1959 on a particular group of organisms, in this case the cellular slime moulds, or Acrasiales. On account of their simple structure and method of fruiting these organisms have proved most useful in the experimental study of fundamental problems of development. Dr Bonner has himself played a notable part in this field and this book is much more than a description of a small but widely occurring group of soil organisms. The first section gives a useful and much-needed comparison of the different groups of organisms possessing a plasmodium or plasmodium-like stage in their life cycles, i.e. Myxomycetales, Plasmodiophorales, Labyrinthulales and Acrasiales. Then follows a detailed review of all that is known (with remarks on what is not known) about the life histories of the various members of the Acrasiales. The second half of the book is taken up by a consideration of two problems of development in the Acrasiales, namely, morphogenetic movement and cell differentiation.

The comprehensive bibliography reveals that more than half the work done on the cellular slime moulds has been reported during the last ten years. Moreover, the group has attracted workers from different biological disciplines, each with his own concepts and methods of approach. In this timely review Dr Bonner has ably brought these views together. As well as being a review of facts, the book is also a personal interpretation of these in the present climate of opinion, including much thoughtful consideration of their possible significance in terms of evolution as well as development in the individual. Although Dr Bonner's assessment may not be accepted entirely by all, it provides a most valuable reorientation on which to base further work.

The style is simple and clear and the author is careful throughout to distinguish between fact and hypothesis. The book is both well printed and attractively produced. Those unfamiliar with the terminology applied to the Acrasiales may notice some omissions from the index, e.g. sorus, sorocarp.

To the microbiologist it is perhaps disappointing that there is no more than a passing reference to the natural habitat of these organisms. All those interested in problems of development both in the Acrasiales and in other groups will find this book stimulating and challenging to read.

G. M. BUTLER

Uredineana. Tome 5. Ed. by A. L. GUYOT. (Paris: Paul Lechevalier, 1959 [*Encyclopédie Mycologique.* Tome 31.].) Pp. 862, with plates and numerous text-figures. Price: 15,000 francs.

This latest instalment (see *Trans. Brit. mycol. Soc.* **37**, 443, 1954) includes 18 original papers (pp. 5–542), an annotated bibliography of important papers (mostly post-1945) on rusts arranged geographically (pp. 543–689) and a further supplement to Guyot's *Les Urédinées*, **1** and **2** (pp. 713–760).

Among the original contributions are papers on rusts of Palestine (by Rayss and Chabelska), Mecklenburg (Buhr), Ivory Coast (Guyot), Pyrenees (Dupias), Tunis, Algeria, and France (Guyot, Chevassut and Massenot). Other papers deal with *Puccinia* spp. on *Crucianella* (Gäumann) and rusts of *Rhaponticum* and *Serratula* (Massenot). [This volume has been added to the Society's library.]

G.C.A.

Bibliographie der Pflanzenschutz-Literatur: 1946–1947. By J. Bärner. (Berlin: Paul Parey, 1957.) Pp. xli + 460. Price: DM. 44.50.

Bibliographie der Pflanzenschutz-Literatur: 1948–1949. By J. Bärner. (Berlin: Paul Parey, 1958.) Pp. xli + 689. Price: DM. 42.

Literaturquellen und ihre Kürzungen aus der Bibliographie der Pflanzenschutzliteratur. By J. Bärner. (Berlin-Dahlem: Biologische Bundesanstalt für Land- und Forstwirtschaft, 1958.) Pp. 167. Price: DM. 9.80.

The last issue of this valuable Bibliography of Plant Protection, covering the years 1950–1951, was published in 1956 (see notice in these *Transactions*, **40**, 176, 1957). At that time, there was a gap in the series over the years 1946–49, caused by the dislocation following the Second World War. The issue of these two volumes now closes this gap in a series that was begun by Prof. Morstatt in 1921, and covers the literature of plant pathology from 1914 onwards. These two volumes come up to the high standards set by their predecessors, and all plant pathologists will wish to congratulate Dr Bärner and his publishers in their successful race against time and an ever-increasing volume of literature.

Maladies of Hevea in Malaya. By Roger N. Hilton. (Kuala Lumpur: The Rubber Research Institute of Malaya, 1959.) Pp. 101, 41 plates in colour by Hoh Choo Chuan. Price M$30.

This handsome book is described in its Foreword as primarily 'a recognition manual for the practical rubber planter in Malaya'. Such grower's manuals are not usually reviewed in these *Transactions*, but there are two good reasons for making an exception in this instance. Some three and a half million acres are now under rubber in Malaya alone, but the mere economic importance of a problem in Malaya does not automatically arouse the interest of mycologists elsewhere. What should arouse their interest is the fact that, in little more than 30 years, the Rubber Research Institute of Malaya has made, through far-sighted planning and continuity of research, a contribution to the knowledge and control of tropical plant diseases that transcends in importance the boundaries of crop and country alike. In the sphere of root disease investigation in plantation crops, research at the R.R.I. of Malaya has led the world, and has won a lasting place in the history of plant pathology.

The mycologist who wishes to follow the progress of current research on diseases of rubber will be no less in debt than the rubber planters of Malaya to Mr Hilton for this admirable and convenient adjunct to his reading. No virus nor bacterial disease of rubber is known in Malaya at present; with the exception of infection by mistletoe and of the unimportant algal leaf spot caused by *Cephaleuros mycoidea*, all the known diseases are either non-parasitic or caused by fungi—a state of affairs liable to arouse nostalgic memories in elderly mycologists. After a brief general introduction on the nature of disease, Mr Hilton treats in turn individual diseases of the whole plant and then those of its parts: the root system, the stem and branches, the tapping panel and the leaves. One page (super Royal 8vo) is devoted to each disease, and the information is classified under common names (English and Malay), symptoms, cause, other plants affected, spread, prevention and treatment. These descriptions are admirably concise, and each faces a full-page water-colour painting of the disease.

The water-colour paintings of all the diseases described are by Mr Hoh Choo Chuan, the official artist of the R.R.I. of Malaya, and provide the second reason for this review. Faithful delineation one has a right to expect, but there is much more than that here. Many of the paintings are of singular beauty and all are a joy to the eye. No botanist nor mycologist who has a genuine love for the plants with which he works can see this book without wishing to possess it. All concerned in its production are warmly to be congratulated—and not least the Kynoch Press, who have reproduced it.

S. D. GARRETT

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Printed in Great Britain at the University Press, Cambridge (*Brooke Crutchley, University Printer*)